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PHYSIOLOGICAL REVIEWS

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PHYSIOLOGICAL REVIEWS
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CHEMOTAXIS IN LEUKOCYTES

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Definition. Chemotaxis is a reaction by which the direction of locomotion of cells or organisms is determined by substances in their environment. If the direction is toward the stimulating substance, chemotaxis is said to be positive; if away from the stimulating substance, the reaction is negative. If the direction of movement is not definitely toward or away from the substance in question, chemotaxis is indifferent or absent.

A. Organisms showing chemotaxis. Chemotaxis occurs in a wide variety of both plants and animals (82). For example, the sperm of ferns is attracted by malic acid, a substance present in the egg cell, as was described by Pfeffer (71) in 1884, in the first paper on chemotaxis. Also by chemotaxis, pollen tubes may be attracted to the ovary (65, 48), and the direction of root growth determined. Myxomycetes (slime molds) react positively to certain carbohydrates (food) and negatively to adequate concentrations of acids and alkalies (17). Protozoa in general show negative chemotaxis, being repelled by injurious concentrations of various substances (41). In metazoa, directional responses to substances in the environment are often mediated by special sense organs, namely, by the sense of smell, which, for example, directs the male moth toward the female, and crabs toward decayed meat. Mammals too may show similar reactions. Hence chemotaxis serves in reproduction, feeding, and avoiding harmful substances. Presumably this directional response to chemical stimuli depends on diverse mechanisms in various plants and animals. In a field so complex, it seems desirable to limit the present review to the chemotactic behavior of leukocytes.

B. Methods. The first recorded observations on chemotaxis of leukocytes seem to be those of Leber (46), who, in 1888, reported results of experiments on rabbit's cornea. Following irritation, leukocytes were seen to move from all directions into the avascular cornea, following straight lines toward the irritated area.

Capillary tube method. In addition, Leber introduced short lengths of capillary tubes into the anterior chamber of the rabbit eye. The tubes contained substances to be tested for chemotactic effect, such as bacteria, which appeared to attract many leukocytes. The capillary tube method enjoyed considerable popularity for some years. A great variety of substances, liquid or solid, were placed in the tubes, which were inserted into the peritoneal cavity (57), anterior chamber of the eye, or subcutaneously (5), or into exudate removed from the body (79). Subsequently the tubes were withdrawn, the contents expressed, and the numbers of leukocytes attracted by different substances were compared. By this method was obtained much information of doubtful validity, for, as demonstrated by Pfoehl (72) and Ruchlädew (76), the method is subject to grave errors. Into the tubes leukocytes enter not only by amoeboid motion, but by floating as well, as

the result of convection currents. These are set up by differences between the liquid in the tubes and that in the tissues outside, differences for example, in osmotic pressure. Under these conditions, erythrocytes as well as leukocytes may be found in the tubes, and since erythrocytes obviously have been carried in passively, the same may be true of leukocytes; therefore their presence is not valid evidence of chemotactic attraction.

Micro-injection method. The uncertainities and errors of the capillary tube technic made a more reliable method desirable, one that should prevent the leukocytes from floating and permit one to make a record of their movements. The experiments of Clark and Clark (10, 11) met these requirements. They injected the material to be tested into the transparent tail of the anesthetized tadpole. With the microscope, leukocytes were seen to emigrate from the blood vessels and then to advance by amoeboid motion toward attracting substances, such as starch grains or a droplet of croton oil. Camera lucida drawings gave conclusive evidence that leukocytes were attracted by several of these test objects. For the *in vivo* method, any transparent tissue, such as mesentery or tissue in the rabbit ear chamber is suitable. Nevertheless the method has been little employed for the study of chemotaxis, perhaps because of technical difficulties.

While speaking of *in vivo* experiments, it should be emphasized that it does not suffice to inject a test material into living tissues, later to sacrifice the animal and to examine the tissues by histological methods. Any even slightly irritating substance thus injected excites emigration of leukocytes, as does, indeed, the injection itself, unless carried out with the utmost precaution, as was done by Clark and Clark. Thus emigration of leukocytes is not valid evidence of a chemotactic response (see below, under Relation of Chemotaxis to Emigration). Rather one must observe with the microscope the direction taken by the emigrated cells, and make sure that they move in more or less straight lines toward the test substance, before one is justified in concluding that the reaction is positive chemotaxis.

Slide-coverslip method. Even before the experiments of Clark and Clark, a simple method *in vitro* was devised by Comandon (21) that met the requirements of direct observation of leukocytes and recording their paths. He allowed a drop of blood to spread between slide and coverslip, and observed with the microscope and recorded in motion pictures the direction of leukocytes relative to the test object. Thus he observed the approach of avian leukocytes to erythrocytes infected with malarial organisms. Also he was able to contrast the positive response to starch grains with the lack of chemotaxis to carbon particles (22).

The slide-coverslip method is suitable for testing the chemotactic effect of solid particles, such as bacteria, carbon, etc., and of tissue fragments. Liquids may be tested if they are adsorbable on particles such as carbon, titanium dioxide or kaolin. The method has the great advantage that individual leukocytes may be observed with the microscope, and their paths of migration relative to the test object may be recorded with the aid of a camera lucida or similar device, as they move toward or away from the test object. One may ascertain whether the cell is advancing by amoeboid motion rather than being carried by convection

currents—(floating is impossible when the suspending medium is blood, or plasma not too much diluted).

Semiquantitative studies of chemotaxis were made by McCutcheon, Wartman and Dixon (55, 23), with the simple slide-coverslip technic. Either whole blood was used, or a suspension of leukocytes in plasma, permitting 10 to 20 cells to be observed in one microscopic field. Suspensions of leukocytes were obtained with the method of deHaan (37), by injecting physiological salt solution into the peritoneal cavity of rabbits and removed the resulting exudate several hours later. The leukocytes, nearly all granulocytes, were then resuspended in plasma, a drop of which was used in making the preparation (52).

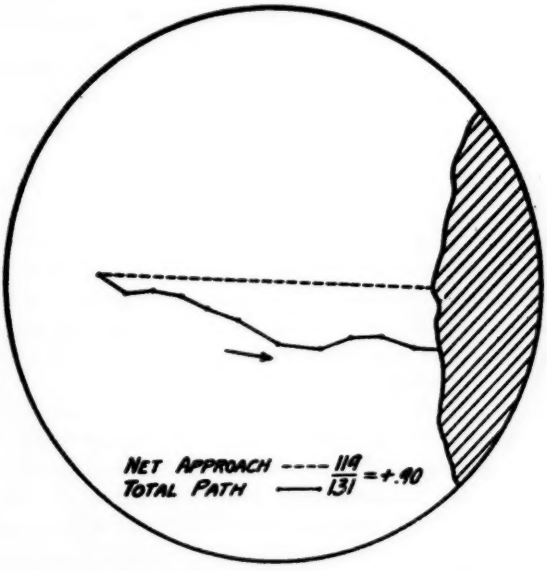

Fig. 1. Method for computing the value of chemotaxis

From a graphic record (fig. 1) obtained with a drawing ocular or camera lucida, the value of chemotaxis may be obtained for each leukocyte by finding the ratio of net approach to total path travelled in the same time. Thus the leukocyte in question was 119 microns from its target at the first observation and 0 micron at the last, hence the net approach was +119 microns. The total path for this time was 131 microns. The ratio is $+119/131 = +0.9$, which is the chemotactic ratio. The limiting values for this ratio are +1.0, if the leukocyte should move directly toward the object, and −1.0 if it moves directly away (23).

If the simplifying assumption is made that 2 lots of leukocytes are moving at the same average speed, their chemotactic responses may be compared merely by comparing their average net approaches. For example, the leukocyte represented in figure 1 approached its object at $119/11 = 10.8$ microns per minute (15).

The tissue culture method. A useful method was worked out by Meier (63), who adapted the plasma hanging drop, as used in tissue culture, to the study of of chemotaxis. Blood is centrifuged and the buffy coat, i.e., the leukocytes, are removed in a layer and introduced into a hanging drop of plasma, which then coagulates. Lymphocytes may be obtained from explants of lymph nodes, macrophages from explants of omentum or other organs. The test object, such as a clump of bacteria, is placed at a known distance from the leukocytes, the reactions of which are then observed with the microscope. If the test substance is in liquid form, it is drawn into a capillary tube, one end of which is sealed, while the open end is placed in proximity to the leukocytes. Leukocytes then migrate from all sides of the explant, but if attracted by the test object, the migration zone is wider on the side of the test object than on the opposite side. The reverse is of course true if leukocytes are repelled.

The resulting distribution of leukocytes may be photographed after one or more hours. By measuring the migration zones with a planimeter, Coman (18) was able to compare the reactions of different kinds of leukocytes to different test objects.

Other methods have generally been less dependable. An *in vitro* method was developed by Jochims (42), who allowed test substances to diffuse through a collodion membrane into a chamber containing leukocytes in liquid suspension. With this apparatus, he reported highly sensitive reactions of leukocytes to differences in H ion concentration. This would be of great interest if one could be sure that the leukocytes were not floating, instead of progressing by amoeboid motion. That is always a danger in liquid medium, unless the cell is under constant microscopic observation.

C. The behavior of leukocytes showing chemotaxis may be studied in the slide-coverslip preparation described above, using either whole blood or exudative leukocytes. For establishing a basis for comparison, the behavior of leukocytes will be described when no chemotactic substance is present. When the preparation is observed with the high power of the microscope the polymorphonuclear leukocytes are seen to spread on the glass and to display amoeboid motion. Locomotion begins almost at once. In the absence of a chemotactic substance, the leukocytes pursue paths that change direction from one minute to another, that is, they move at random. The rate of locomotion varies with the temperature (51) and with unknown factors affecting individual cells; under good conditions, human polymorphonuclear leukocytes at 37°C were found to move at an average rate of about 34 microns per minute (50).

To demonstrate chemotaxis in such a preparation, a minute clump of bacteria, such as staphylococcus, is placed on the glass slide and allowed to dry. Then a drop of blood is superimposed and made to spread between slide and coverslip. When the polymorphonuclear leukocytes in the same microscopic field as the bacteria are observed, practically all are seen to move toward the bacteria, not in a straight line but in a path that deviates a little to one side or the other, often becoming more nearly straight as the leukocyte approaches the bacteria (55). Reaching the bacteria, the cells almost or quite cease moving as they begin phagocytizing.

The chemotactic field: its width. If microscopic fields are examined at increasing distances from the bacteria, the percentage of leukocytes moving toward the bacteria becomes less. Thus, of leukocytes distant 0.4 mm. or less from the bacteria, 89 per cent moved toward the bacteria in a given minute, while of leukocytes 0.5 to 0.8 mm. distant, only 70 per cent did so. Further away a field was reached in which only approximately 50 per cent of cells moved toward the bacteria, that is, motion was here at random. In the set-up described, the width of the zone of attraction was about 1 mm. (54). Compared with this distance Comandon (22) found a zone of attraction about a starch grain of at least 0.2 mm.

The chemotactic field: its time relations. There are two other points of interest about chemotaxis under the conditions described. First, chemotaxis is usually of maximal intensity as soon as the leukocytes begin to move, within a minute or two; that is, almost all the cells close to the bacteria move toward them in nearly straight lines. Little diminution in chemotaxis may be noted for 2 hours or more. Second, the width of the zone of attraction does not increase with time but remains about the same (54). These two observations are consistent with the supposition that an attracting substance is washed off the bacteria when the preparation is made, and is spread to an effective distance of about 1 mm., and that the substance is non-diffusible. However, the chemotactic effect of bacteria or other particles cannot be diminished by repeated washings in distilled water. Presumably the merest traces of the attracting substance are enough to elicit maximal chemotactic response.

The rate of locomotion is not affected by chemotaxis; thus, leukocytes move no more rapidly when responding to chemotactic stimuli than when moving at random (25). For example, in one series of experiments, leukocytes moving toward *Staphylococcus albus* travelled at the rate of 28 ± 7.6 microns per minute, whereas leukocytes too far away from the bacteria to show any chemotactic response moved at least as fast, 32 ± 7.4 microns per minute. Thus chemotaxis does not affect the rate of amoeboid motion; rather is chemotaxis a directional response superimposed on amoeboid motion.

D. The kind of leukocyte showing chemotaxis is chiefly the polymorphonuclear leukocyte (granulocyte), and this cell has been observed to give the response in mammals, birds (21) and amphibia (14). In man, the neutrophile is the type of granulocyte studied most, though eosinophiles also show chemotaxis.

Ingraham and Wartman (39) studied chemotaxis in *eosinophiles* of a man who had leukemia, and also observed eosinophiles from bullae of a patient with dermatitis herpetiformis. Blood eosinophiles from the leukemic patient, observed by the slide-coverslip method, moved toward *Staph. aureus* at the rate of 9.0 microns per minute, this rate being not significantly different from that of neutrophiles of a normal man (7.5 microns per minute). Eosinophiles from bullae of the patient with dermatitis herepetiformis showed somewhat less response, probably because these leukocytes had degenerated. As eosinophilia is often excited by animal parasites, these authors observed the reaction *in vitro* of these cells to *Trichinella spiralis*. The organism was dried and ground, and

used as a test object with the slide-coverslip technic. This material attracted eosinophiles from the leukemic patient no more strongly than it attracted neutrophiles from a normal person. The values were respectively 5.8 and 7.0 microns per minute, and the difference was not significant. Rosegger (75), too, observed chemotactic responses in eosinophiles.

Lymphocytes do not show chemotaxis to any substance yet tested (14). The difference between lymphocytes and polymorphonuclear leukocytes in this respect was evident where these two types of cells were observed in the same field of the microscope (23). In slide-coverslip preparations polymorphonuclears were attracted by various bacteria, but lymphocytes moved at random, toward or away from bacteria, often changing direction. Thus toward *Staph. albus*, the chemotactic ratio of polymorphonuclears was +0.65; of lymphocytes, 0.00. In the presence of tubercle bacilli values were respectively +0.58 and −0.10 (the latter not significantly different from 0.00), nor were lymphocytes attracted by caseous material from tuberculous lungs (24).

Similar results were obtained by Coman (18) in tissue culture by planting staphylococci, tubercle bacilli or clumps of aluminum silicate in plasma, a millimeter or two from a fragment of rat lymph node. As lymphocytes migrated into the plasma, their direction of locomotion was not affected by the bacteria or silicate; they formed a migration zone as broad on the side facing these test objects as on the opposite side. Under these same conditions, polymorphonuclears were attracted by the bacteria and repelled by aluminum silicate.

In the tissues, lymphocytes migrate from blood vessels much as do polymorphonuclears (12), and cells generally regarded as lymphocytes accumulate about tubercles, malignant tumors, transplants, and in chronically inflamed tissues. Such accumulations of lymphocytes suggest that they may have been attracted chemotactically. Though this may be true, other explanations are possible: lymphocytes may have wandered into position through random movements, but, once there, may be prevented from leaving; they may have multiplied locally, or cells resembling lymphocytes may actually be degenerated polymorphonuclears (14).

Chemotaxis in monocytes. Since these cells are the most efficient of all mammalian cells as phagocytes, it might be confidently expected that they would show positive chemotaxis. Observations in fixed and sectioned tissues support this expectation. For example, when tubercle bacilli are injected in animals, monocytes accumulate about them as if attracted chemotactically. Similarly in pneumococcic pneumonia of man and experimental animals, monocytes regularly move into the pulmonary alveoli in great numbers shortly before recovery, and there apparently play a decisive part in terminating the infection (Robertson, 74). Further, direct observation of living amphibian tissues has given evidence that monocytes react chemotactically (14).

It is astonishing, therefore, that *in vitro*, mammalian monocytes appear to show only weak chemotaxis or none at all. When bits of rat omentum were explanted into plasma, and clumps of bacteria were placed nearby, monocytes migrated promptly from all sides of the explant, but failed to show chemotaxis, as the

migration zone was of equal thickness on all sides (Coman, 18). Under the same conditions, polymorphonuclears showed an asymmetrical migration zone, which was broader on the side toward the bacteria, giving evidence of positive chemotaxis. When the test object was aluminum silicate, the migration zone of monocytes was narrower on the side facing the test object than on the opposite side, giving evidence of negative chemotaxis; but the effect was only slight; while statistically valid, there was actually only slight repulsion, in contrast to strong negative chemotaxis shown by granulocytes under the same conditions.

Recent experiments by Jacoby (40) suggest that monocytes may in fact show positive chemotaxis *in vitro*, but that the attracting influence extends only a short distance. When monocytes were planted in serum and adhered to the glass bottom of a Carrel flask, a cell here and there showed death changes. Photographs of the culture showed that neighboring monocytes, to a distance of about 25 microns, converged from all sides on the degenerated cell, this response ending in phagocytosis of the dead cell.

The question as regards chemotaxis in monocytes is not completely answered. From present evidence it seems likely that chemotaxis plays a smaller part in the activity of these cells than it does in polymorphonuclear leukocytes.

E. Substances that excite chemotaxis. As chemotaxis is a directional reaction to substances in the environment, it is now appropriate to inquire what substances or particles excite this reaction.

I. *Microorganisms. Bacteria* are the most important source of attraction, as chemotaxis to bacterial products aids in combatting infection. Apparently bacteria in general excite positive chemotaxis, though under conditions to be mentioned presently, the reaction may be inhibited or masked. When tested by the slide-coverslip method, human and rabbit granulocytes were attracted by a variety of bacteria including pyogenic cocci such as staphylococci, streptococci, pneumococci, as well as by such diverse microorganisms as typhoid, tubercle, colon and Welch bacilli (53). An exception was noted in certain strains of beta hemolytic streptococci, which either failed to excite chemotaxis, or else possibly repelled the leukocytes (52). Heat killed bacteria attracted leukocytes as well as did living ones (33). In these experiments, leukocytes were observed while in close proximity to bacteria. If, however, there was a greater distance, one or two millimeters, between leukocytes and bacteria, leukocytes were not attracted by some bacteria, such as M. tuberculosis, but were attracted by Esch. coli, staphylococci, pneumococci and streptococci (60). Thus the distance over which attraction occurs depends on the particular microorganism, perhaps being greater in the case of rapidly growing bacteria than in the slowly growing acid-fast organisms. Dead bacteria at a distance of 1 or 2 millimeters from leukocytes excited no chemotactic response.

In vivo, bacteria in general give off chemotactic substances, but the effect of these is not infrequently masked by other and poisonous bacterial products, which retard or stop locomotion of leukocytes, so that the cells are unable to reach the bacteria. Consequently the number of granulocytes that collect about bacteria in the tissues varies widely. Moreover, some bacteria, such as the

pyogenic cocci, attract leukocytes for as long a time as the bacteria grow—in staphylococcic osteomyelitis this may be for years—whereas more slowly growing organisms such as *M. tuberculosis* attract granulocytes only at certain times. Thus when tubercle bacilli were injected into the blood stream of animals, granulocytes collected where the bacilli lodged, and this happened within 24 hrs. Afterwards these cells were replaced by monocytes. But again after a week or two when the tubercle becomes necrotic, a second wave of granulocytes surrounded the bacteria (Lurie, 49; Woodruff, 87).

Toxic bacterial products and "negative chemotaxis." Reference has been made to the crippling effects on leukocytes of substances produced by many bacteria. In typhoid fever granulocytes are generally absent from the lesions; this is probably the result of poisoning of the bone marrow, so that maturation of granulocytes is prevented and the number of these cells in the blood is reduced. In *Clostridium Welchii* (gas bacillus) infections, leukocytes are paralyzed by a soluble toxin produced in the lesion; consequently leukocytes are unable to approach the bacteria (29). Similar effects may be produced by virulent strains of staphylococci (Stevenson and Reed, 81) streptococci and other microorganisms.

The fact that virulent bacteria may inhibit reactions of leukocytes has important consequences. Such infections, unchecked by phagocytic cells, tend to spread rapidly and to destroy life. Indeed virulence may signify chiefly that certain strains of bacteria produce substances that inhibit the activity of leukocytes. Inhibition of leukocytic movement may, however, be overcome by the presence of antibodies (70), or may be prevented by previous immunization of the animal (57). In these ways, toxic bacterial products are neutralized.

Similar effects of toxin-producing bacteria have been noted *in vitro*. An early investigator of chemotaxis, Massart (57), inserted capillary tubes intraperitoneally; if these tubes contained virulent microorganisms, few leukocytes collected in them, in contrast to great numbers of cells in tubes containing non-virulent bacteria. Recently, the effect of different strains of staphylococcus was tested on the migration of cells from explants of mouse spleen. In the presence of virulent strains, the migration zone was narrower than when less virulent strains were tested (81). Morgan and Upham (67) found that typhoid bacilli retarded migration of cells from an explant of guinea-pig spleen, as compared with the effect of *Staph. albus*, while a protein-free antigen of *E. typhosa* was toxic enough to inhibit migration.

Such interference with the motility of leukocytes, preventing them from approaching and phagocytizing bacteria, has usually been attributed to negative chemotaxis. This is probably incorrect, and is due to misunderstanding of the term "negative chemotaxis." As defined above, negative chemotaxis is the reaction of moving away from a substance in the environment. If leukocytes showed negative chemotaxis to certain bacteria, one might expect to be able to demonstrate this reaction *in vitro*, but thus far no definite negative chemotaxis to bacteria has been so demonstrated. It seems more likely that bacterial poisons injure leukocytes, impairing their power of locomotion. Excessive numbers of bacteria in a culture of leukocytes may soon cause migration to cease

(King, Green and Henschel, 43). Bacterial substances that kill leukocytes have been called leukocidins.

How do bacteria produce their chemotactic effects? There are two possibilities: first, bacteria may produce substances in the course of metabolism that attract leukocytes; second, bacteria may injure tissues, which then give off attracting substances. *In vivo* it is difficult or impossible to separate these two processes, and probably both occur at the same time. *In vitro*, however, tissue damage may be entirely avoided, so that under these conditions, chemotaxis is a response to bacteria directly (26). Even so, under these experimental conditions there is another possibility, that bacteria may attract leukocytes by means of substances, such as polypeptides, absorbed from the culture medium. That this is not necessarily true was shown by Meier (61), who used no artificial culture medium but grew bacteria in plasma, then freed the plasma of bacteria by filtration, and showed that the plasma had acquired the property of attracting leukocytes.

As to the nature of this metabolic product, it is probably a protein or a heat stable (Grand and Chambers, 35) protein derivative. That bacterial proteins are chemotactic has long been known (7). The molecular size of the attracting substance, as indicated by its filterability, is of the same order as proteins (61), and its lack of diffusibility, mentioned earlier, is consistent with its being protein. From staphylococci, Menkin (64) obtained an attracting substance having the properties of polypeptides, which are protein derivatives.

Several fractions of the tubercle bacillus were studied by Wartman and Ingraham (83, 84) for their chemotactic properties. Tubercle protein, when tested in its undissolved form, failed to attract leukocytes, but when dissolved and adsorbed on charcoal, excited positive chemotaxis, the index being +0.69. Tubercle phosphatide was toxic to leukocytes but when adsorbed on titanium dioxide elicited weakly positive chemotaxis (index +0.43). A polysaccharide from tubercle bacilli was found to induce negative chemotaxis in leukocytes of both man and rabbit. In each case the index was −0.34.

Somewhat similar results were obtained with fractions of hemolytic streptococci (27). Little or no attraction was produced by a carbohydrate fraction adsorbed on kaolin (index +0.18) nor by a protein-free, non-antigenic hemolysin (index −0.03). An antigenic protein-carbohydrate complex elicited positive chemotaxis (index +0.64), but this result was not confirmed by later (unpublished) experiments, so the effect of this fraction remains in doubt.

Viruses. There is no direct evidence as to whether or not viruses attract leukocytes, but it is inferred that they do not, as granulocytes are usually few or entirely absent in tissues infected by viruses, provided there is no associated infection by bacteria. The intracellular location of viruses during multiplication may account in part for their failure to attract granulocytes.

Protozoa. The organism of avian malaria was observed by Comandon (21) to excite strong positive chemotaxis. Leukocytes moved directly toward parasitized red cells and pushed them along until they burst. The leukocyte might then move toward the nucleus of the red cell, but more often it followed the

parasite and ingested it along with grains of hematin. The leukocytes were said to move as fast as 1 to 2 microns per second, and seemed to travel more rapidly as they neared the parasitized cell, though no measurements were given to support this statement.

Yeasts. *Torula histolytica* causes little inflammation and attracts few leukocytes in tissues. *In vitro*, leukocytes were attracted at least for a short time (53).

II. Chemotaxis excited by *products of injured tissue.* It is well known that exudation of leukocytes into tissues may be caused by a variety of injuries, even in the absence of infection. Thus large numbers of leukocytes may be found in sterile blebs resulting from burns (Moon 66), as well as in irradiated tissues (9), and may be attracted by injecting various intensely irritating substances such as turpentine. These effects are generally believed to be due to chemotaxis, an explanation supported by experiments *in vitro*. Thus leukocytes were accumulated in capillary tubes containing products of tissue injury (58); they were attracted by dried leukocytes (55) and by a thermolabile substance released by injured muscle fibers (35). Silverman (80) investigated attracting substances in injured human skin, and found that pulped skin excited strong positive chemotaxis (index +0.83). The attracting substance was soluble in water. After extraction with water, pulped skin no longer attracted leukocytes (index +0.05), but from the aqueous extract the attracting substance might be recovered by adsorption on kaolin (index +0.81). This substance was thermostable and gave reactions for protein.

Histamine. Among products of injury, histamine was early thought of as a substance possibly attracting leukocytes, because several of the processes found in inflammation may be produced by injection of histamine.

Wolf (86) made chambers with agar impregnated with histamine, filled the chamber with 2 drops of blood, and subsequently counted the number of leukocytes adhering to the agar. She reported a strongly positive chemotactic effect as compared with control chambers. Others have failed to confirm this effect of histamine. Bloom (6) found no attraction of leukocytes in capillary tubes containing histamine. Grant and Wood (36) injected histamine into various tissues, but failed to obtain a leukocytic response. Moon (66) implanted pith, impregnated with histamine, into the peritoneal cavity, but no more leukocytes were attracted than when the pith contained only serum. After extracting the chemotactic factor from skin pulp, Silverman (80) impregnated the pulp with histamine. With the slide-coverslip method, the chemotactic ratio was +0.25. Histamine adsorbed on kaolin yielded a ratio of −0.13. Silverman concluded that there was little if any chemotactic response.

In contrast to the predominatingly negative results *in vitro*, histamine may cause abundant emigration of leukocytes from blood vessels. This result was observed by Abell (1), who introduced histamine into the moat of the rabbit ear chamber. It is possible however, that the emigration of leukocytes was not a chemotactic reaction, but rather a reaction to histamine-induced changes in vascular endothelium. This possibility is discussed below.

Leukotaxine. Menkin (62) produced sterile exudates in dogs and obtained by fractionation a thermostable, dialyzable substance having the properties of polypeptide. This substance, which he called leukotaxine, attracted leukocytes both in tissues and *in vitro*. A substance with similar physiological properties was obtained by tryptic digestion of serum proteins (63). This work was confirmed by Duthie and Chain (28), who obtained by digestion of fibrin a polypeptide that excited positive chemotaxis.

Thus injured tissues are an important source of chemotactic material, and the reaction is advantageous, eliminating damaged tissue through phagocytosis and digestion by leukocytic enzymes.

III. *Carbohydrates.* Grains of starch attract leukocytes both in tissue and *in vitro*. When starch grains were injected into the transparent tail of the tadpole, leukocytes emigrated and moved toward the grains, which were then phagocytized and digested (11). Chambers and Grand (8) placed starch grains in capillary tubes and observed that leukocytes from the buffy coat of fowl's blood and from the spleens of chick embryos and mice were attracted to and entered the tubes, clustering in masses about the starch grains until these were digested. Comandon (22) too, observed positive chemotaxis to starch. Also other polysaccharides excited positive chemotaxis, notably glycogen (8) and agar (14, 8). Threads of raw cotton were found to attract leukocytes to the cut end, but not to other parts of the thread, as if attracting substance escaped from the cut end. Silk threads, on the contrary, were not chemotactic (8). Chambers and Grand regarded attraction by polysaccharides as due to products of serum amylase digestion, and in support of this explanation reported that polysaccharides were not chemotactic in the absence of serum, and also that various sugars attracted leukocytes. Sheldon (78), using the method of Chambers and Grand, confirmed attraction by starch and glycogen but not by sugars. Chemotaxis to carbohydrates appears to be a feeding reaction, just as is slime molds, which are attracted by various carbohydrates (17).

IV. *Substances exciting negative chemotaxis* in leukocytes. Considerable confusion exists in the literature as to the use of the term negative chemotaxis, as many workers mean by it absence of chemotaxis, while others mean a repelling effect, the opposite of positive chemotaxis. The term should be used only in the latter sense, to signify a reaction by which the leukocyte moves away from a substance, that is, moves from higher to lower concentrations.

The possible negative chemotactic effect of certain bacteria was mentioned in a previous paragraph, where it was stated that no clear cut example of negative chemotaxis to bacteria has yet been recorded, though Wartman and Ingraham (84) reported negative chemotaxis to tubercle carbohydrate.

A preparation of aluminum silicate (marketed as Lloyd's reagent) induced negative chemotaxis in human and rabbit leukocytes (52). In slide-coverslip preparations, practically all the leukocytes in the field of the microscope were seen to move away from the particles in almost straight lines. Under these conditions, exudative rabbit cells moved away at the average rate of about 7 microns per minute, the reaction being about as strong as, but in the opposite

direction to, positive chemotaxis to bacteria under similar conditions (20). The significance of this repelling effect of silicate (not shared by silica) is not understood. It is astonishing that the only substance thus far shown to cause strong negative chemotaxis in leukocytes is one which the cells would not be likely to encounter naturally. Myxomycetes also are repelled by Lloyd's reagent (17). The subject of negative chemotaxis in leukocytes needs further investigation.

V. *Chemotactic effects of particles* having low solubility in water. The repelling effect of aluminum silicate has just been noted. Collodion particles have the opposite effect, strongly attracting leukocytes (25). Both these substances have low solubility in water, and their effects on leukocytes are not lessened by repeated washing in distilled water. It is remarkable that particles having these properties should excite any reaction at a distance. In the case of collodion, these particles are known to adsorb proteins readily, and it may be they had become contaminated with minute amounts.

Most other fairly insoluble particles—silica (54), carbon (15), titanium dioxide (84),—exert no chemotactic effect under the best controlled conditions. Hence chemotaxis to these particles is absent. Manganese dioxide was reported by Fenn (30) to excite positive chemotaxis. In slide-coverslip preparations, he observed that leukocytes moved toward the MnO_2 particles, although they showed no reaction to quartz particles which they passed on the way. Quartz and carbon particles were encountered only by chance. Chambers and Grand (8), on the contrary, found no attraction to be exerted by MnO_2.

VI. *Miscellaneous substances.* Chambers and Grand (8) found that physiological solutions, hyper- and hypotonic solutions, and solutions of various salts did not excite chemotaxis. Olive oil, oleic and stearic acids, sodium and potassium palmitate were indifferent substances. As several of the substances mentioned are strongly surface active, these investigators dissented from the view of Schade (77) and of Häbler and Weber (38) that chemotaxis depends on the surface activity of the exciting substance.

Hydrogen and hydroxyl ions. Jochims (42) reported that solutions between pH 5,8 and 6,3 attracted leukocytes, between 4.4 and 4.9 were negatively chemotactic, and from 6.9 to 8.5 were indifferent. These results cannot be unreservedly accepted without confirmation, as Jochims used liquid medium in which leukocytes might have been transported passively by convection currents. Gräff (34), too, thought chemotaxis to be dependent on a hydrogen ion gradient, but other workers have found no effect of hydrogen and hydroxyl ions on chemotaxis (80). In this connection it is interesting to note that myxomycetes (slime molds) exhibit negative chemotaxis to adequate concentrations of both hydrogen and hydroxyl ions (17).

In the older literature, reviewed by Wells (85), are found reports on the chemotactic effects of a wide variety of substances. These will not be reviewed in the present paper.

F. Conditions interfering with or lessening chemotaxis are important from the standpoint of the defense against infection, since, if cells cannot be attracted,

their arrival in an area of infection will be delayed, and thus bacteria have a better opportunity to become established. As already stated, bacteria of high virulence may inhibit the motion of leukocytes. While the effect of poisoning of leukocytes is chiefly on their rate of locomotion, there is evidence from other types of experiment that such poisoned cells show also decreased chemotaxis. Thus in the case of individuals who were acutely ill from a variety of causes, their leukocytes in vitro showed both decreased rate of locomotion and less directional response to bacteria (56).

In acute experimental intoxication with alcohol (Klepser and Nungester, 44), the same functions of leukocytes were depressed: the rate of locomotion was decreased and the cells, in the presence of pneumococci, showed no chemotactic response. With the slide-coverslip method employing whole blood of rats, the chemotactic index in leukocytes from normal animals was +0.55; in cells from animals comatose from alcohol, the value was +0.06. Thus chemotaxis was abolished. When alcohol was added directly to human blood *in vitro*, the chemotactic index fell from a control value of +0.46, to +0.24 when the concentration of alcohol was 0.1 per cent, and to +0.16 with an alcohol concentration of 0.4 per cent. Such effects of alcohol may be responsible in part for the decreased resistance to infection occurring in alcoholic intoxication.

G. Relation of chemotaxis to emigration of leukocytes. Emigration of leukocytes from blood vessels is widely regarded as the result of chemotaxis, in the sense that a concentration gradient of an attracting substance exists between inflamed tissue outside the vessels and the leukocyte inside the vessel, and as the result, the leukocyte moves through the vessel wall into the outside tissue. Against this explanation is the fact that one of the best ways to cause leukocytes to emigrate is to inject a physiological salt solution into a serous cavity. Although such a solution when tested *in vitro* has no chemotactic effect, it causes vast numbers of leukocytes to collect in serous cavities. Thus emigration apparently may occur without chemotaxis. But, it should be added, if the salt solution contains a chemotactic substance, even greater numbers of leukocytes emigrate (16). As another example of emigration due to factors other than chemotaxis, lymphocytes have been observed to emigrate from vessels (12), although they are apparently incapable of displaying chemotaxis. A clearer understanding of the factors in emigration is gained from the experiments of Clark and Clark (13), who studied the reactions of leucocytes in the transparent tail and fin of the tadpole, and in the transparent chamber set in the rabbit ear. If slight mechanical pressure was made on a blood vessel, leukocytes were seen to adhere to the endothelium for a short time, and then usually were carried away by the blood stream. Sticking appeared to result from reversible changes in the vascular endothelium. Greater pressure, or injury to the surrounding tissues, however produced, was followed by adhesion of many cells to the vessel wall, and by morphological changes in the lining cells that were interpreted as softening. Some of the leukocytes adherent to the endothelium then passed through the vessel wall by amoeboid motion. Thus emigration was always preceded by changes in the endothelium, interpreted as increased stickiness and

as softening, so that emigration appeared to result not from chemotaxis but from endothelial changes. According to Baron and Chambers (3) the leukocytes, too, become sticky.

Various other mechanisms have been proposed to explain emigration of leukocytes (45), all depending on differences in physical or chemical factors between the blood inside vessels and the tissues outside vessels. These factors have been thought to be the pressure exerted by the blood on the vessel wall, higher concentration of hydrogen ions (31) outside the vessels than inside, or greater viscosity of tissues as compared with blood (32). All these hypotheses lack confirmation, as does the more recently expressed view of Abramson (2) that emigration may be brought about by differences in electric potential between the injured area and the blood. From evidence already presented it seems likely that emigration is conditioned first by changes in endothelium with consequent stimulation of leukocytes to display amoeboid movement and to pass through the vessel wall; second, emigration is probably increased by chemotaxis.

H. The mechanism of chemotaxis. The generally accepted explanation of the phenomena of chemotaxis in leukocytes is the surface tension theory, as developed by Wells (85). He regarded the leukocyte as a drop of viscous liquid, which tends to take a spherical shape. If one side of the cell should be exposed to some substance that lowers tension at the surface of the leukocyte, the cytoplasm would flow out in that direction and the cell move. Thus the cell is regarded as a passive drop of liquid, under the control of surface forces, a conception that arose as the result of experiments with artificial cells (73). A number of cell models have been contrived which imitate certain aspects of amoeboid motion. Thus if a drop of mercury is surrounded by a dilute solution of nitric acid, and a crystal of potassium dichromate is placed near the drop of mercury, chemical changes occur especially on that part of the surface of the mercury nearest the crystal, and the drop of mercury suddenly and rapidly moves to the crystal and flows around it. Thus positive chemotaxis is simulated (4). If the mercury is placed in a solution of potassium dichromate, and nearby is placed a drop of concentrated nitric acid, the mercury is impelled to move in the opposite direction, that is, away from the nitric acid, and thus negative chemotaxis is simulated (19).

This conception of the leukocyte as a drop of liquid behaving passively is not in accord with present day conceptions of the structure and mode of motion of the cell, for amoeboid motion is now explained by most workers as the result of reversible sol-gel transformations of the cytoplasm. The forward end of the cell is regarded as chiefly in the sol state, and this sol becomes transformed to gel at the surface as the cell moves. The rear end of the cell is mostly in the gel state; the gel is transformed to sol, which is pushed forward by contracting jelled cytoplasm, either because the gel is under tension (59) or because it contracts much as does muscle (47). Thus the motion of the cell is not passive but active, involving transformation of chemical energy into kinetic.

Chemotaxis is a directional response superimposed on amoeboid motion as just outlined. In fact, amoeboid motion takes place just as well without chemotaxis

as with it, the rate of locomotion not being increased by the chemotactic response (25). What chemotaxis does is apparently to determine where in the cell shall occur solation and where gelation. How this is done is unknown. Evidently the cell is able to detect differences in concentration of the exciting substance at the one end of the cell and at the other. Polymorphonuclear leukocytes at least, are able to detect this difference when they lie in a concentration gradient of a chemotactic substance, whereas lymphocytes, at least according to present information, lack a mechanism for responding to differences in concentration of substances in their environment.

If the simple surface tension theory of chemotaxis were correct, there should be closer correspondence between chemotaxis and phagocytosis than we find. Thus there is evidence that phagocytosis is promoted by, or, indeed, may be dependent on certain surface active substances, namely, antibodies (68). These are thought to coat bacteria and to lower the tension at the leukocyte-bacterial interface, so that the leukocyte is able to spread upon and to engulf the bacterium. Here is an example of the effect of surface active substance upon phagocytosis. If chemotaxis also were under the influence of surface active substances, then antibodies should increase chemotaxis, but available evidence is opposed to this supposition. When leukocytes were observed by the slide-coverslip technic, and their direction was followed in the presence of streptococci, specific antibodies were found not to increase chemotaxis to the bacteria, whether the leukocytes were attracted strongly or weakly, whether the antibody was adsorbed to the bacteria or was contained in the plasma medium (20).

It is concluded that the weight of evidence is against the surface tension explanation of chemotaxis, as it was elaborated by Wells. On the other hand, no complete and satisfactory alternative theory has as yet been proposed.

I. The relation of chemotaxis to phagocytosis. The idea seems to be held by many pathologists that phagocytosis is always preceded by chemotaxis, that is, before a bacterium can be engulfed, it must attract the phagocyte from a distance. This is certainly not correct. Many particles, especially of inorganic material such as carbon and silica do not attract leukocytes though they are frequently phagocytized by them as the result of chance encounters (30). This may readily be observed in slide-coverslip preparations, where polymorphonuclear leukocytes may accidentally make contact with such particles and may then ingest them. Monocytes, the most voracious phagocytes, are, at least in mammals, not definitely known to be attracted to the bacteria and cells that they engulf (18). Thus it is clear that phagocytosis is not necessarily preceded by chemotaxis. Conversely, chemotaxis is not always followed by phagocytosis, as was seen when polymorphonuclear leukocytes were attracted by dead cells, droplets of cream and of egg yolk, yet did not engulf these objects (14). A still more impressive example of chemotaxis not followed by phagocytosis is the negative, i.e., repelling, effect of silicates, described above (52).

The mechanisms of phagocytosis and of chemotaxis appear to be quite different. Phagocytosis depends on the ability of the leukocyte to spread on the object to be phagocytized, and spreading is greatly aided by specific antibodies known as

tropins (68). Tropins, as noted above, appear to play no part in chemotaxis, which is apparently a non-specific reaction excited often by antigens, but not, so far as is known, by antibodies (20).[1] Chemotaxis is not essentially a spreading reaction, like phagocytosis, but a reaction that depends on complicated sol-gel transformations in different parts of the cell body.

J. Of what advantage is chemotaxis of leukocytes to the organism and under what circumstances does this reaction occur? It has generally been accepted that leukocytosis, i.e., increased numbers of leukocytes in the blood, depends on chemotactic attraction of leukocytes from the bone marrow into the blood stream. For this supposition there seems to be no evidence. It has likewise been held by most pathologists that emigration of leukocytes from blood vessels into tissues is the result of chemotaxis. This has already been discussed, and the situation has been summarized by saying that both chemotaxis and changes in vascular endothelium probably play a part. Chemotaxis is chiefly important as the reaction by means of which leukocytes are attracted to infecting microorganisms from a distance. It is especially the polymorphonuclear leukocytes that are so attracted. These cells constitute the first line of cell defense. They are mobilized quickly and in great numbers. By chemotaxis they are directed in more or less straight lines toward bacteria, and thus much less time is required to bring these leukocytes into actual physical contact with the microorganisms. This contact is followed, if the bacterial surface is suitable, by phagocytosis and intracellular digestion. By means of these successive activities of the leukocyte, infection may be overcome. Chemotaxis, phagocytosis and intracellular digestion are thus consecutive activities of the leukocyte depending on different mechanisms.

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[1] Opie (69) found that the precipitate formed by interaction of horse serum and anti-horse rabbit serum is chemotactic for leukocytes when injected into the skin of the rabbit.

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THE PHYSIOLOGY OF NERVOUS SYSTEMS OF INVERTEBRATE ANIMALS

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The basic metabolic functions of an animal can proceed without any highly specialized conducting tissue. The specialization of conducting tissue, particularly of nervous tissue, gives an animal a certain freedom from the environment which has survival value; this freedom permits a combination of large size and high motility. Many Protozoa and larvae are small and have little rapid conduction from one part to another, yet are motile. Many sessile animals, on the other hand, have slightly developed conducting mechanisms; they may be large but they are not motile. All of those animals which are both large and motile have rapidly conducting nervous tissue.

In the evolution of nervous tissues four trends are apparent: (1) increasing speed of conduction, (2) integration, or making one impulse count for more or less than one, (3) cephalic dominance, and (4) chemical mediation of nerve impulses. The present review aims to discuss the attainments of invertebrate animals in each of these directions of nervous evolution and to point out useful preparations for physiology rather than to chronicle the literature on invertebrate nervous systems. The most recent review of this subject was by Ten Cate in 1931 (301).

PRE-NERVOUS CONDUCTION. Among the animals which do not have nervous systems some co-ordinating mechanisms have been described. Probably all living cells have the ability to conduct waves of excitation, and conduction in many plant cells, eggs and body cells of higher animals has been described as a wave of depolarization passing along the plasma membrane. Conduction from cell to cell, as in ciliated epithelium, is well known. Conduction by way of many intracellular fibrils has been claimed for a few Protozoa.

Taylor recently reviewed the protozoan fibrillar systems (292). Many ciliates have a regular network of fibrils connecting the basal granules of the cilia. The form of this network varies, but in general the basal granules are connected in longitudinal rows with some cross-connections (115 and others). This peripheral network is connected to the central neuromotor system which may have a center or motorium and has internal fibrils which extend to membranelles, cytopharynx, and cirri where these are present. The entire fibrillar system has been variously claimed to be supporting, contractile and conducting. Experimental evidence of a conducting function came from Taylor (291), who with microneedles cut fibrils between the motorium and the cirri in Euplotes (and cut the peripheral fibrils as well); co-ordination between the cirri and membranelles in the oral region disappeared. Disruption of co-ordination of membranelles occurred after destruction of the motorium in Chlamydodon (188), and disorganization of ciliary movement after fibrillar injury in Ichthyophthicus (190). However, in Paramecium lesions near the peristome did not

destroy ciliary co-ordination (204), even though a motorium is said to be located there (187).

Conduction in higher animals is a function of cell membrane and even in neurones the fibrils per se probably have nothing to do with conduction. Taylor (292) suggests that the fibrillar system in ciliates actually may have several different functions. Ciliates are highly specialized acellular organisms. Hence, the ciliate fibrillar system is not a precursor of nervous structures.

In sponges there are no nerve cells, but spindle-shaped muscle cells bring about closure of oscula. These muscle cells Parker (229) has called independent effectors because they combine sensory and motor functions. There is a slow conduction (about 1 cm./min.) from cell to cell in a sponge as shown by the response of the osculum to a prick a short distance away (223). In some coelenterate tentacles there may be a few two-celled arcs (sense cell—muscle cell) (229), but beginning with the coelenterates co-ordination between sense organs and effectors is essentially nervous.

NERVOUS CONDUCTION. *Action Potential Wave.* Impulses in nerve fibers of invertebrate animals consist, as in vertebrates, of several electrical components. The non-medullated fibers of the squid (6) and crab (136) have been used for the demonstration of non-propagated, graded, local potentials. From the local potential emerges the propagated spike, which is followed by after-potentials. In crab nerve a negative "retention" may continue for fifteen minutes after a stimulus (182, 110). Zhukow (337) described an after-positivity which might persist for a similar time. Veratrine caused the after-negativity in crab fibers to continue for a half-hour (79, 27), and the after-negativity was lengthened by repetitive stimulation (46). These invertebrate nerves provide excellent preparations for analysis of the cellular basis for after-potentials.

Speed of Conduction. Five different adaptations of nerves have provided increased speed of reaction among animals: length of nerve processes, fiber diameter, myelin sheath, nodes, and giant fibers.

(*1*) *Length of nerve-processes.* The first nervous adaptation by which animals increase their speed of reaction is the lengthening of conducting processes. If a message has to pass through numerous synapses, with a delay at each, it travels more slowly than if it can go by way of a single fiber. In many coelenterates, for example, the nervous system consists of a network of multipolar and bipolar neurones whose processes have been traced for several millimeters (50). Conduction in this network is slow, less than 0.5 m./sec. (table 1). In some coelenterates through tracts of continuous fibers exist for rapid conduction (221). In general, fast-moving animals have some nerve tracts consisting of long single fibers. Giant fiber conduction is more rapid than conduction through the neuropile in ganglion chains of annelids and arthropods (table 1).

(*2*) *Fiber diameter.* In mixed nerves of frogs and mammals speed of conduction is proportional to a function of the fiber diameter. The fiber-diameter/velocity correspondence has not been investigated for the entire spectrum of any invertebrate nerves. However there is no doubt that faster animals have some large motor nerve fibers, and in slower animals most nerve fibers are small.

In some mammalian fibers the velocity in meters per second numerically equals the diameter times a constant (6 or 7) (113, 149), in frog fibers the velocity varies as the square of the diameter (94), and in giant fibers of the squid velocity increases as the diameter raised to the 0.61 power (251).

TABLE 1

Velocity of conduction in ganglionic cords and nerve nets

ANIMAL	REFERENCE	TEMPERATURE	VELOCITY
Ganglionic cords			
		°C.	m.p.s.
Annelids			
Earthworm	(181)		0.6
Leech	(181)		0.4
Earthworm (Helodrilus)	(48)		0.02 -0.03
*Cerebratulus	(155)		0.059–0.09
*Arenicola	(155)		1.2
*Aphrodite	(155)		0.545
Myriapods			
Scolopendra ant. → post.	(62)		2.5
Scolopendra post → ant.	(62)		2.5
Julus ant. → post.	(62)		0.2
H mantarium ant. → post.	(62)		0.28
Crustacea			
Cambarus	(243)	20–24	1.2
Nerve nets of coelenterates**			
Metridium	(228)	21	0.121–0.146
Calliactis (net)	(221)		0.04 -0.15
Calliactis (through tracts)	(221)		1.2
Physalia filaments	(231)	26	0.121
Cassiopea	(126)	18	0.136–0.234
Cassiopea	(199)		0.15 -1.2
Mastigias	(170)	25.5	0.57
Renilla (muscular wave)	(230)	21	0.078
Renilla (luminescent wave)	(230)	21	0.074

* These are a few of the numerous values given by Jenkins and Carlson (155). Some of the species studied are now known to have giant fibers and some of the higher velocities they recorded may have been in giant fibers.

** Most conduction velocities in coelenterates are measured as speeds of contractile waves rather than as conduction in nerve net only.

Table 2 gives the velocities in moist air and where possible the total fiber diameters in a variety of nerve fibers. The general relationship between diameter and velocity holds. The motor fibers of the legs of crustacea for example are 20–30 μ in diameter and conduct at 1.5–12 m./sec., while molluscan fibers about 1 μ in diameter conduct at less than 1 m./sec. The most striking adaptation of

TABLE 2

Velocity of conduction in representative nerve fibers as related to fiber diameter and sheath thickness

ANIMAL	NERVE	REFERENCE	FIBER DIAMETER (MICRA)	AXIS CYLINDER DIAMETER/FIBER DIAMETER	TEMPERATURE	VELOCITY
Warm-blooded vertebrates						
Dog	saphenous	(113)	17		38	83.3
Dog	saphenous	(295)	16	0.82		
Dog	saphenous	(113)	14.4		38	71.5
Dog	n. lingualis	(113)	13.5		38	69.1
Cat	saphenous	(114)	13	0.71	38	80
Cat	saphenous	(149)	12–15		37.5	65–82
Cat	saphenous	(114)	9	0.69	38	60
Dog	chorda tympani	(113)	8.2		38	36.4
Dog	saphenous	(114)	5	0.6	38	20
Cat	saphenous	(295)	2	0.3		
Rabbit	depressor	(217)	4–2		38	5
Cat	sympathetic	(42)	thin	non-myelinated	38	2–1
Cold-blooded vertebrates						
Frog	dorsal root	(113)	18.5		21.5	42
Frog	dorsal root	(113)	14			25
Frog	dorsal root	(113)	11			17
Frog	dorsal root	(113)	5.8			4.2
Frog	sciatic	(274)	6–16	0.7		
Frog	sciatic	(274)	2–3	0.5		
Frog	B fibers	(42)	small	thinly myelinated		4.5–3
Frog	dorsal root (C fibers)	(113)	2.5	non-myelinated		0.5–0.4
Turtle	sympathetic & vagus	(42)		non-myelinated		0.8–0.3
Invertebrates						
Arthropods						
Maia	leg nerve	(186)	10–20, 4–8, 1–3		23–25	2.5–3.7 1.1–1.75 0.1–0.5
Carcinus	leg nerve	(137)	30	very thin sheath	21	3.5 in oil
Giant crab	leg nerve	(165)				2.7, 3.9
Callinectes	leg nerve	(205)				4.8, 1.5
Munida	leg nerve	(142)	50	very thin sheath	17	6.4
Homarus	leg nerve	(205)				9.2, 1.8
Homarus	leg nerve	(63)				12
Cambarus	leg nerve	(88)				4.2, 3
Leander		(142)	<10	0.53		
Leander		(142)	10–20	0.69		
Limulus	leg nerve	(205)				4.6, 1.3
Cockroach	cercal nerve	(250)				5–6, 1.5
Cockroach	central fibers	(256)	10–20	0.9–0.95		
Molluscs						
Helix	n. intestinalis	(178)	(<1 ?)			0.4, 0.05
Ariolimax	pedal	(154)			15–18	0.41
Limax	pedal	(154)				1.4
Pleurobranchiae	pedal	(154)			12–15	0.78
Mytilus	pedal	(258)				0.64
Sepia	mantle	(46)	<50			3.5, 2.26
Sepia	fin	(46)				5.1
Sepia	stellar	(7)				3, 1.5, 0.32, 0.22
Loligo		(251)	42.6 avg.		21–22	4.6 avg.
Loligo	fin	(154)				4.3

TABLE 2—*Concluded*

ANIMAL	NERVE	REFERENCE	FIBER DIAMETER (MICRA)	AXIS CYLINDER DIAMETER/ FIBER DIAMETER	TEMPERATURE	VELOCITY
			Giant nerve fibers			
Vertebrate						
Ameiurus	Mauthner fibers	(117)	22–43	0.59–0.56	10–15	50–60
Arthropod						
Cambarus		(319)	200–250	(median)		
			60–90	(lateral)		
Leander		(142)	35	0.77	17	18–23 in oil
Munida		(142)	50	thin sheath	17	6.4
Shrimp		(294)	>35	0.87		
			<8	metatropic		
Annelid						
Lumbricus		(293)	>40	0.90		
			<11	metatropic		
Lumbricus		(89)	median		10–12	17–25
			lateral		10–12	7–12
Lumbricus		(288)	median 64			
			lateral 37			
Lumbricus		(58)	median			15–45
			lateral			5–15
Lumbricus		(48)				1.5
Lumbricus		(269)				20, 10
Mollusc						
Sepia		(251)	147			7.7
Loligo		(251)	718			22.3
			589 avg.			20.6
			335 avg.			13.7
Loligo		(276)	280–400		22	20
"		(29)	400–500	>0.99 metatropic		

fiber diameter to velocity of conduction is in the giant fibers of crustaceans, cephalopods and annelids. These may have diameters of tens of micra or several hundred micra and conduct many times faster than the smaller fibers of these animals. Insects in general have very small nerve fibers but conduction distances are short, hence fast conduction is less important than a large number of fibers in a small volume. Richards, for example, states (256) that mosquito larvae fibers are less than 1–2 μ although cockroaches have some 10 μ fibers.

Excitation times also vary inversely with fiber diameters, i.e., fast fibers have short chronaxies.

(*3*) *Nerve fiber sheath.* In addition to fiber diameter the existence of a lipoid sheath around the axon is directly correlated with velocity of conduction. Among vertebrates the larger fast motor and sensory fibers have myelin sheaths which blacken with osmic acid and can be readily measured, while the slow small sympathetic fibers are said to be non-myelinated. When examined in polarized light the larger fibers are seen to be negatively birefringent with respect to their long axis; this is due to the lipid sheath and the fibers are called myelotropic. The birefringence in frog fibers is nearly constant among fibers 9 μ or more in diameter but diminishes in smaller fibers until it is zero at 2 μ and below that it reverses sign (274). The birefringence of the "non-medullated" fibers on the contrary is normally positive with respect to length, is due to proteins, and can

be counterbalanced by immersion in glycerine of the same refractive index, whereupon a slight negative birefringence (with respect to length) appears; these fibers are said to be metatropic.

Most invertebrate nerve fibers are said to be non-myelinated but in many of them examination with polarized light reveals a thin outer layer which is positively birefringent with respect to length (28, 275). Immersion in glycerine shows certain fibers of crabs, shrimps, cockroach and other insects, Nereis, etc., to be metatropic (256, 294, et al.). The protein layer has a refractive index of 1.58 in fibers from lobster cord, frog motor root and cat corpus callosum (69). The reversal in sign in glycerine reveals a thin lipoid layer which in insects may even stain with sudan dyes (255). This layer can be removed with fat solvents. As the diameter increases the positive birefringence decreases so that earthworm giant fibers are weakly myelotropic above 11 μ and shrimp giant fibers above 8 μ (294) as compared to 2 μ in frog fibers (274). Squid giant fibers are uniformly positive with respect to length regardless of diameter, but when immersed in glycerine a lipoid sheath is demonstrated which is less than one per cent of the fiber diameter (29). In summary, except for large giant fibers of earthworm and shrimp most invertebrate fibers resemble vertebrate non-myelinated fibers in optical properties—a thin lipoid sheath masked by protein.

The fastest vertebrate fibers are of medium size (4–15 μ) but have thick sheaths (30–50 per cent of total fiber diameter) whereas the fastest fibers of invertebrates are large (50 to several hundred micra), but have thin sheaths (1–10 per cent of the diameter). In both groups the ratio of axis cylinder to total fiber diameter usually increases with larger fibers up to some critical size. Taylor (295) has summarized the relation between diameter, sheath and velocity as follows: a 4 μ fiber of the cat saphenous at 38° conducts at about the same rate (2 m./sec.) as a 650 μ squid giant axon at 20°. Assuming a Q_{10} of 1.5, an 8 μ cat saphenous fiber would conduct at 20° at the same rate as the 650 μ squid giant fiber. The myelin sheath of the mammalian fiber constitutes some 42 per cent of its total diameter whereas in the squid giant fiber the sheath is about one per cent of the fiber diameter.

Many invertebrate nerve fibers also have a connective tissue sheath. In some (prawn, 141; squid, 336) connective tissue cells lie between the thin lipoid sheath and the axon. There is no evidence that the connective tissue sheath speeds conduction.

How fiber size, relative sheath thickness, and birefringence influence speed of conduction is a problem of cellular physiology about which there is little but speculation.

(*4*) *Nodes.* Erlanger and Blair (93) obtained evidence that impulses in frog medullated nerve travel in a saltatory fashion from node to node. There are regions of thinning of the sheath in shrimp giant fibers (141, 142). Dependence on sheath and nodes for speed is, however, largely a vertebrate adaptation.

(*5*) *Giant fibers.* There are two types of giant fiber systems. Each has apparently evolved independently several times. The first type consists of a single large or giant cell which gives rise to a single large axon. The second

type consists of a syncytium in which processes from a number of nerve cells fuse to form a single giant axon. Unicellular giant fibers are found in nemerteans and cestode worms, in numerous polychaetes, in balanoglossids and in lower vertebrates. In the polychaete Halla for example (8) are groups of unipolar giant cells 30–150 μ in diameter in each of the first few segments; these give rise to fibers up to 40 μ thick which may run the length of the worm. Similar unicellular giant fibers have been described in other worms. Bullock (57) and Hess (135) have described the giant fiber system in a number of balanoglossids. Typically there are about a dozen to 150 giant cells, usually in the collar nerve cord; these give rise to large (3–6 μ) fibers which run back in the nerve cord and eventually dwindle in size to merge with the ordinary fibers ($< 1\ \mu$). The only functional studies on the unicellular type of giant fiber are on the Mauthner and Müller cells which occur in the tegmental nuclei (part of the vestibular reflex system) of fishes and amphibians. These fibers, 22–43 μ in diameter, conduct at 50–60 m./sec. at 10–15°C (table 2) (117).

The multicellular or syncytial type of giant fiber system has been described in annelids, crustacea and cephalopod molluscs.

In the ventral nerve cord of the earthworm there are in each segment several cells which send axones to the three dorsal giant fibers, one large median and two smaller lateral ones. These giant fibers run the full length of the ventral nerve cord and give off segmental branches to motor neurones in the cord. The median giant fiber is connected to two pairs of giant cells and each lateral giant to one pair of giant cells per segment (289). The lateral giant fibers are also connected with each other. In addition to the three dorsal giant fibers there are two smaller ventral ones (288, 280). At each segment there is in the giant fibers an oblique partition the two sides of which stain differently (289, 58). This was thought by Stough to be a macrosynapse.

Evidence from cutting the giant fibers, from regeneration, from blocking by drugs, and from embryonic development indicates that in the earthworm the giant fibers are responsible for quick end-to-end "startle" contractions (48, 239, 303, 289). Stough (289) cut single giant fibers and concluded that the median one conducts postero-anteriorly while the two lateral ones conduct antero-posteriorly. Action potentials (89), however, showed that conduction is equally good in either direction in each fiber. Bullock (58) settled the disagreement by showing that the median fiber is normally excited by sensory stimulation in the anterior forty segments while the lateral fibers are excited by sense organs behind that level. The two lateral fibers and the median fiber form two separate conduction and excitability systems; cross connections between the lateral fibers were shown by conduction which continued when right and left fibers were alternately cut (269). The usefulness of the giant fiber system particularly in protective defense reactions is indicated by the fact that conduction in the smaller fibers of the cord (including synapses) is about 0.025 m./sec. (48) while in the lateral giant fibers it is 7–12 m./sec. and in the median one it is 17–25 m./sec. depending on fiber size (89). Multicellular giant fibers occur also in Nereis and in many other polychaete annelids.

Among Crustacea giant fiber systems have been described in the crayfish, the lobster, and in the prawns Palaemonetes and Leander. In the crayfish (157) there are three kinds of giant fiber: median, lateral and motor. The paired median giant fibers start in the brain (at least in the crayfish) decussate there and pass to the ventral nerve cord. The lateral fibers occur in thoracic and abdominal ganglia and consist of segmental units which appear to overlap in long side-by-side contacts; they presumably are made up of processes from ganglion cells in each segment. Branches are given off from the laterals and medians to motor giant fibers in each segment. The motor fibers have their cell bodies in one segment, decussate, and pass out in the posterior nerve of the segment (157). Stimulation of the giant fiber system elicits quick flipping of the abdomen (158) and causes extension and inward movement of antennae (319). Johnson (157) thinks that the median ones conduct backward, the lateral ones forward. We have found (Prosser and Altschul, unpublished) that the median and lateral giant fibers of the crayfish conduct equally well in both directions. Wiersma (319) found summation between the two median giants to be maximum at an interval between stimuli of 1.7 msec. and between the lateral giants at less than 4.5 msec.; he noted some summation of the response of the median by stimulating the lateral giants and concluded that the four fibers govern the same nerve-muscle system.

In the prawn Leander, Holmes (141) described two types of synapse, oblique regions of apposition between the lateral fibers in each segment and typical boutons at the points of contact of processes from both median and lateral with the motor fibers. The median giant fibers showed incomplete septa. Transection of the cord at various points failed to produce degeneration of the median or lateral giant fibers. In Leander the motor giant fibers are non-myelinated from the cell body to their synapse and are myelinated from that point out to the muscles. The lipid sheath of the larger giants is interrupted occasionally (142) and is separated from the axon by a thin nucleated layer and does not have such a layer outside it as do the fibers in vertebrates. The ratio of axis cylinder to total fiber diameter is 0.53 for fibers smaller than 10 μ, 0.69 for 10–20 μ fibers and 0.77 for 20–50 μ fibers (142).

The cephalopod molluscs have a giant fiber system which is intermediate between the unicellular type and the segmentally fused type of giant fiber system. The cephalopod system has been studied by Young (335, 336). In the decapods (squids) a pair of giant cells lie at the posterior end of the pedal ganglion portion of the brain, their processes fuse and cross, then synapse in the visceral part of the brain mass with giant cells whose processes run out in mantle nerves. These synapse with a third set of giant fibers in the stellate or mantle ganglion. From the stellate ganglion some dozen nerves pass out to the muscles of the mantle; each nerve contains one giant fiber which may be as much as 800 μ in diameter plus many one-micron fibers. Each giant axon arises by the fusion of processes from 300–1500 cells in the stellar ganglia and the preganglionic axon ends by synaptic boutons near the region of fusion. Each giant axon serves the circular muscles of a large area of the mantle and the largest fibers pass to the posterior

part of the mantle so that impulses arrive nearly simultaneously in the entire mantle (336). Velocities of conduction in fibers 30–718 μ in diameter range from 2.7 to 22.8 m./sec. at 20°. As shown in table 2 there is a very thin sheath. Stimulation of a giant fiber elicits a maximal contraction at threshold (336), and there is no increase in tension with increasing frequency of stimulation (248), hence the one syncytial fiber with a large area of circular muscle is a motor unit. The contractile response forces water out of the mantle cavity thus sending the animal backward; this escape reaction is, according to Young, twice as fast by virtue of the giant fiber system as it would be without it.

Young (335) has described an interesting series among cephalopods. In the decapod Sepia the cells contributing to the large giant fibers are scattered through the stellate ganglion. In Loligo these cells are confined to one posterior lobe, while in the octopods Eledone and Octopus the lobe is present and contains cells which may resemble neurones but which lack true processes. This lobe, the epistellar body, receives the secondary giant fibers from the visceral ganglion; that it may be a neurosecretory organ was indicated by an atonia of the mantle after removal of the epistellar body.

Giant fibers have been very useful preparations for studying the membrane mechanisms of nerve conduction, and Curtis and Cole (80) have successfully recorded impulses with a fine electrode inserted down the center of a squid giant axon. This method gives more nearly the total membrane potential than does the usual surface recording.

Giant fibers, then, are usually adaptations of large fiber diameter for rapid escape reactions. Some are connected to one cell, some are syncytial. The syncytial annelid and crustacean fibers show either segmental septa or long areas of apposition which appear to conduct equally well in either direction. Such junctions certainly do not behave physiologically in the same way as most synapses in higher animals. The marked differences in structure and function indicate that giant fibers have been evolved independently in several groups.

Co-ordination. Among the co-ordinative functions which are associated with the vertebrate central nervous system are polarity of transmission, spatial and temporal summation, after-discharge and inhibition. These, except for polarity, permit a single impulse to count for more or less than one. Among invertebrates much co-ordination occurs at neuromuscular junctions as well as in central nervous systems, and it is useful to consider both sites as parts of a common system.

Nerve nets. Co-ordinative functions evolved very early; they are found among nerve nets where there is no condensed central nervous system. Nerve nets or diffuse nervous systems have been studied functionally in coelenterates, both polyps and medusae and in ctenophores. The elements of the nervous system of medusae were identified by Haeckel and the Hertwigs, and were described by a series of early histologists as a network of multipolar and bipolar neurones lying beneath the epithelium and connected to a condensed marginal ring. Bethe (37) and Hadzi (121) contended that the nerve fibers anastomose and that the network is a syncytium. Parker (229) also accepted this view. Recent

evidence (50, 330) with vital stains shows that processes of the neurones often intertwine or run along parallel with one another and may have a beaded appearance while in contact but that the fibers do not fuse. These "synapses" are not structurally polarized like vertebrate boutons but the nerve net is probably not a syncytium.

Coelenterate nerve net systems show some co-ordinative characteristics.

1. *Polarity.* The first indications in medusae were of diffuse, non-polarized conduction (91, 266, 267). The marginal "sense organs" are the normal pacemakers for rhythmic contractions. Conduction is diffuse and essentially equal in all directions. If overlapping lateral and central incisions are made, or if the jellyfish is cut into a long zig-zag strip, or into a central disc connected by a narrow neck to an outer ring, impulses pass around corners and in both directions so long as nervous tissue is present. That this diffuse conduction is nervous is shown by conduction through areas where muscles are absent, but nerve fibers present (200), in regenerating areas where conducting but not contracting tissue is present and in preparations treated with curare (266) or magnesium (267, 200, 56). A doughnut-shaped ring can be cut out of a jellyfish and a wave started in both directions from a stimulus at a point (199, 126); if one wave is blocked in some way, e.g., by pressure on one side, a circusing self-perpetuating wave can be set up which keeps going around for many hours (up to several days); the same units are being re-excited repeatedly.

Parker (227) made similar observations of diffuse conduction on sea anemones. He made cuts of various shapes in the body wall and found that conduction went through various types of connection from the body wall to the muscles. Similar conduction occurred from zooid to zooid in the colonial Renilla (230).

It is concluded from the effects of cuts in the nerve net and from the histological picture that there is no polarity in conduction from neurone to neurone. Final evidence can come only when conduction across a single junction can be examined.

The picture of a non-polarized diffuse conducting system is, however, too simple for all the facts. Parker (227) showed that the oral disc of Metridium responds differently according to whether the tentacles are stimulated by acid or by mussel juice, indicating either specific functional tracts or differences in sensory response. Pantin (222) showed that when a tentacle is removed from an anemone (Calliactis) or pieces cut from a tentacle the end of the central stump contracts while the end of the free piece remains open. Also it is easier to get impulses out to the tentacles than in from them, i.e., less facilitation is needed. Pantin (221) also found some through conduction, especially in the mesenteries where velocities of 1.2 m./sec. existed in contrast to local conduction around or up the anemone at 0.04–0.15 m./sec. Through tracts were more important in Calliactis than in Anemonia. Also the velocity of local conduction varied greatly in different regions. This evidence from anemones indicates definite conduction pathways for different purposes and a sort of functional polarity. Pantin indicated that this polarity may arise by the development of differential rates of facilitation.

2. *Facilitation.* It was formerly believed (162) that nerve net conduction is decremental because a contraction wave dies out with increasing distance from the stimulus. Pantin has accounted for the decremental-like conduction in sea anemones in terms of facilitation (220). He found that an anemone rarely showed any muscular response to a single shock but gave an increasing contraction as more stimuli were delivered. At low frequency (intervals greater than 2 sec.) a response might be seen only after 10–15 stimuli at 20°. Increasing the intensity of successive shocks had little or no effect upon the contraction but increasing the frequency greatly increased the response to a maximum at 100–200 msec. separation between stimuli. The second of two stimuli was effective to 5 per cent of the maximal response at 5° when separated from the first by 8 seconds, at 15° at 4.6 sec. and 24° at 0.9 sec. intervals (122). Each impulse excited some units subliminally, distant ones less than near ones, and new units were brought in as excitation built up. The excitatory process persisted longer at low than at high temperatures. Thus the response spread further and became stronger as more distant units were brought up to threshold.

Bethe described facilitation in a tissue bridge in a medusa (39) and Bullock has studied facilitation in medusae in detail (56). Jellyfish differ from anemones in that single stimuli elicit a small contraction. Bullock found enhanced responsiveness to last longer than 20 seconds in some medusae. Facilitated twitches also showed enhanced relaxation. Whether the facilitation observed in the nerve net system is myo-neural or neuro-neural is not critically important in terms of the overall integrative properties of the system. The nerve net system showed a long refractory period—0.7 sec. absolute and 0.1 sec. relative in the jellyfish; smooth fusion of response to repeated stimuli never occurred. The long refractory period probably belongs to the muscle. Ross and Pantin (268) found Mg to block the facilitation. They also observed an increase by potassium. Bullock (56) confirmed the Mg effect and showed that during the period in which neuromuscular block gradually becomes complete there is a stage when contractions change from general to local responses; these barriers can be broken by repeated stimulation, hence they are apparently due to depressed neuro-neural facilitation. Certainly the increased response with increasing frequency is not due to increased contractility (staircase). Bullock presented evidence that facilitation is important in determining strength of contractions in normal rhythmic activity of jellyfish.

3. *After-discharge.* Pantin (222) observed that as responses build up during repeated stimuli supernumerary responses frequently occur. These may represent a sort of after-discharge.

4. *"Inhibition."* We mentioned Mayer's blocking of a wave of contraction in a jellyfish ring by pressure. Two opposing waves of luminescence in sea fans cancel out when they meet (206), and analogous phenomena have been reported in ctenophores (68). No evidence for periods of depressed excitability, however, has come from the use of paired or repetitive stimulation of anemones or medusae. The cancellation of colliding waves may be due to refractoriness of conducting pathways. In a jellyfish ring preparation Kinosita (170) showed that if the

absolute refractory period of the nerve elements (0.25 sec. at 24.5°, 0.8–0.62 sec. at 15°) exceeded the circulation time the wave stopped. The absolute refractory period of the nerve lengthened less with cooling than did that of muscle so that above 16° the refractory period of nerve was longer and below that temperature that of muscle was longer.

Cancellation of a wave by pressure raises the possibility that some of the net conduction is reflex in nature, i.e., that contracting muscles re-excite conducting fibers. The possibility of local reflex arcs in the net system seems not to have been explored. There are reciprocal contractions of circular and radial muscles in jellyfish (49) and possibly of muscles of the two sides of a flat ctenophore (210) which may be activated by separate nets acting in reflex co-ordination, although Bozler (49) suggested that in Scyphomedusae each of the different nerve layers may serve special sets of reflexes.

Present evidence indicates, then, that coelenterate nerve net systems demonstrate a polarity due to (1) discrete anatomical pathways and (2) differences in facilitation for conduction in a given direction; that they show facilitation following one stimulus at both myoneural and neuro-neural junctions, i.e., the necessity for repeated impulses to produce a threshold excitation; that facilitation may lead to supernumerary discharges; and that evidence for any true inhibitory state is lacking.

Peripheral Versus Central Control of Locomotion. Among coelenterates the peripheral network is the nervous system. A subepidermal nerve network exists in many groups—echinoderms, balanoglossids, annelids and molluscs. Jordan (162) has called those animals in which behavior is under peripheral control the "hohlorganartigen Tieren." Jordan and his students think of the peripheral network in invertebrates as being analogous to autonomic ganglia, centers of distribution of certain centrally originating messages. The present discussion, on the other hand, inquires to what extent the peripheral system can act independently and considers that its relay function is of secondary importance. It is appropriate to compare locomotion in many invertebrates with movement of the mammalian intestine in which the nerve network is essential for co-ordinated movements even though the muscle may be capable of spontaneous movement. The subepidermal nervous system and the central nervous system are in competition with respect to locomotor control. In general in more sluggish animals the peripheral system is more important and in more active forms central reflexes take over locomotor reactions. Even in animals where locomotion is entirely centrally controlled, visceral plexi remain autonomous, e.g., the myenteric plexus of the vertebrate intestine and the stomatogastric system of annelids and arthropods.

The peripheral portion of the nervous system is more important for locomotion in echinoderms and in balanoglossids than in any of the worms, molluscs or arthropods. The competition between peripheral and central nervous components is of phylogenetic interest.

The nervous system of echinoderms consists of a central oral ring from which the radiating ambulacral nerves originate. These radial nerve trunks contain

cell bodies and connect by branches to the peripheral network which innervates tube feet, spines, etc. (282). The peripheral network contains ganglion cells, for example in Echini in rings at the base of large spines. The peripheral nerve network can conduct impulses independently of the ring and radial nerves. Jennings (156) cut through a radial nerve of a starfish and found a prick at the tip of the injured ray to cause pedicellariae of other rays to rise. He also found that in a piece of the dorsal body wall of a starfish stimuli in any part excite all of the pedicellariae on the piece. If one arm of an ophiuroid is placed under tension, excitation of the ring goes to the stretched arm rather than to other arms (310). Similar results were obtained with fragments of sea urchin shells in which spine movement in response to NaCl stimulation was studied (171). A piece of paper soaked in NaCl caused a nearby spine to bend toward the stimulus; if a cut was made between the paper and the spine there was no response. If one spine was pulled a nearby one was stimulated to bend and if now a cut was made between the two the excitation spread from one to the other, provided a third spine was at the end of the cut. If a spine was held so that it could not move, waves were not conducted past it. These results indicate that the ganglion cells at the base of each spine constitute local reflex centers, and that the peripheral system is not a continuous network.

The starfish central ring has a directive function. A starfish with radial nerves cut eventually rights itself but shows poor co-ordination (72). If all elements of the ring were removed, an isolated arm travelled toward its base, if a bit of the ring remained, the arm moved toward its distal end; if the ring was cut in two places, the starfish pulled itself in two (147). Langeloh made comparable observations with Antedon (180); he concluded that echinoderms show no fixed central control but a "plastic reciprocal relation between peripheral conditions and central functioning." In two monographs Smith (282, 283) has described the interrelations between peripheral plexi, radial and ring nerves as a series of reflex arcs.

In general, the central ring is directive, the radial nerves are necessary for true locomotion and righting, but alone the peripheral system can permit a certain amount of co-ordination between pedicellariae, spines, etc.

The nervous system of balanoglossids (Enteropneusta) consists of a subepidermal plexus together with dorsal and ventral cords and collar connectives (55). Experiments in which the cords were cut showed that the subepidermal network can conduct both circularly and longitudinally but conducts more readily circularly. Conduction in the proboscis is entirely by way of the subepidermal network. The dorsal, ventral and collar trunks seem to be essentially conducting cords and show little integrative activity.

In tunicates a single ganglion lies between the two siphons. Experimental work has been done largely on Ciona and Ascidia, in which a peripheral nerve net has not been adequately demonstrated. When the ganglion is removed or cut transversely, intersiphonal reflexes are abolished (128, 129, 169, 81, 97, 298, 9). Strong protective reflexes are also abolished. Local responses of a single siphon and of the body to mechanical stimuli remain but reaction time is

increased, and excitability diminished. Opinions are equally divided as to whether tonus is increased or decreased by extirpation of the ganglion. Day (81) slit a siphon and observed that a contraction wave was conducted around the cut. It seems certain that a direct connection exists from epidermal receptors to underlying muscles in ascidians, but conduction and tonus properties of the muscle need elucidation.

Among higher chordates peripheral nerve networks are retained only for co-ordination of smooth muscle movement, particularly in the gut. On the other side of the phylogenetic tree (worms—molluscs—arthropods) peripheral nets are less important in controlling locomotion than on the echinoderm-chordate side.

Among flatworms dependence upon central neurons is striking in view of their primitiveness in other respects. In free-living flatworms the nerve trunks (2 to 8 in number) run posteriorly from the brain and contain nerve cells which are not grouped into ganglia. As long as a portion of one of these nerve cords is left in a piece of a planarian, spontaneous movement and some co-ordinated responses persist (23). Eggers observed (90) this necessity of a bit of remaining cord also among Nemerteans. The only example of independent function of the peripheral system is in the planarian proboscis where if the two connections to the central nerve trunks are severed the proboscis autoamputates and shows food-seeking reactions (168).

Annelids, being tubular and containing a movable coelomic fluid, might well be expected to show peripheral co-ordination. In the earthworm a subepidermal plexus of branching fibers and scattered nerve cells lies outside the circular muscles; each central ganglion gives rise to three pairs of segmental nerves containing both sensory and motor fibers which connect with the subepidermal plexus. Can this peripheral network conduct impulses along the body? Can a strip of earthworm body wall move spontaneously? Is there connection from epidermal sense cells through the peripheral network directly to muscles?

It has long been known that if the ventral nerve cord is cut or is removed from the earthworm for several segments peristaltic waves still pass from one end of the worm to the other. Peristaltic transmission occurs if the two pieces of a transected worm are connected by a thread or when a few segments are anesthetized (101, 102). Coonfield (76) found that if part of the nerve cord was removed a wave of mucus secretion failed to pass from normal to denervated segments. If the nerve cord is removed from more than three segments and those segments are firmly pinned down so that no pull can be exerted beyond them, no peristaltic wave passes but if less than three segments are denervated and these segments pinned down a wave is conducted (241). Action potentials in the segmental nerves show that each nerve serves three segments, the different nerves serving overlapping fields which are fairly discrete. When one nerve is stimulated there is contraction in its own segment and to a less extent in adjacent segments (241). Janzen (151) made a T-shaped cut in the lateral body wall of an earthworm; he observed transmission of a contraction wave "around the corner" if the nerve cord was intact, but not in a strip lacking the nerve cord.

Spontaneous contractions have been seen in strips of earthworm body wall

lacking the ventral nerve cord (109, 290, 125, 151, 127) and have been denied (54, 48, 143). An earlier suggestion that spontaneous activity exists in suspended denervated strips but not in non-suspended strips (239) seems not to hold. In segments from which the nerve cord had been removed both muscle layers remained tonically contracted; this was ascribed (143) to a peripheral plexus which normally was inhibited by the central nervous system.

Janzen (151) obtained spontaneous periodic contractions in strips which had been narcotized during dissection but not in non-narcotized strips; the contractions were usually local but could be propagated; the two muscle layers could contract separately. Hatai (127) reported spontaneous activity in dorsal strips from the posterior end of Allobophora but not from the anterior portion and not at all from Perichaeta. We have made similar observations on Lumbricus. The muscles of segments anterior to and including the clitellum often remained contracted at the edges for hours after excision; this was probably a response of the smooth muscle to injury and not evidence for peripheral ganglia as Von Holst (144) had suggested; these excised segments failed to show spontaneous movements. Post-clitellar strips relaxed but were not spontaneously active. Dorsal strips consisting of about twenty segments just in front of the posterior tip, however, showed spontaneous contractions in four out of seven preparations. It appears then that posterior regions of the dorsal body wall are capable of spontaneous movement but there is no reason to implicate the subepidermal plexus. Janzen's drug experiments are not convincing. The body wall muscle may, like the smooth muscle of the mammalian intestine, be capable of some intrinsic movement. Conduction through a sheet of smooth muscle is known and likely occurs in the separate layers of earthworm muscle.

Janzen (151) found that tactile stimulation of dorsal body strips elicited contractions, usually local but sometimes propagated; spontaneously active strips showed the best responses. We have noted similar effects and obtained contractions both when the tactile stimuli were applied to the muscle side and to the skin side of the strip. The responses resemble those of a guinea-pig ileum to prodding. The body wall muscle may be responding to direct stimulation. However, in a few of the preparations from the posterior end a clear-cut response to bright illumination was obtained after dark adaptation. It is probable that in the response to illumination and to very light touch there is some direct connection between sense endings and muscle. However, the weakness and the local nature of the response and its virtual absence in anterior segments show that it can hardly be of importance in behavior.

The preceding evidence indicates that the subepidermal plexus of the earthworm is not a true nerve net for general conduction, that while there may be connections through it from sense cells to muscle these cannot be of importance in behavior and that there is no evidence that the network plays any part in initiating spontaneous movement of the body wall. The peripheral plexus is probably a sensory relay area since there are many more epidermal and muscle sense cells than there are segmental nerve fibers (241). It is possible that among more primitive annelids the connection through the peripheral plexus from sense

organs to muscle was important but that in earthworms the central nerve cord has now taken over complete control. Certainly among polychaetes (e.g., Arenicola (164)) there is no evidence of any conduction of peristaltic waves except by way of the nerve cord.

In gastropod molluscs a subepidermal plexus has been described, particularly in the foot (41, 37). Biedermann (41) found that small pieces of the foot of a slug could show peristaltic movement whereas pieces of the foot of Helix did not show such movement. Jordan (159, 161) removed the paired pedal ganglion from Aplysia and noted a marked increase in tonus of the "wings;" this was arbitrarily attributed to a release of the peripheral net from inhibition by the pedal ganglion. When ganglionic connections to the "wings" of Aplysia were cut (138) and one of the motor nerves was stimulated, there was an immediate contraction of the area served by that nerve followed by a peristaltic wave which spread out from this area. This wave was said to be reflex in nature with the peripheral network conducting the impulses. Cobb (71) found that excised labial palps of Anodonta show autonomous responses to stimulation by light, touch and chemicals. These palps must contain direct connection through the peripheral nervous network from sense organs to muscle. In general, molluscan subepidermal networks may relay messages from central ganglia but are incapable of any independent locomotor control. There is little doubt that normal co-ordinated locomotion of the foot requires the presence of the pedal ganglia. More knowledge of the conduction properties of the muscle is needed before functional importance can be assigned to the peripheral network.

The use of a peripheral nerve network in locomotion appears with slight success in the echinoderm-chordate line and with still less success in the annelid-arthropod-mollusc line. In the higher groups—vertebrates, polychaetes, cephalopod molluscs, and arthropods there is no evidence of a peripheral network for locomotor control. Yet in most of these a nerve network is retained for regulation of movement in the digestive system. Central nervous reflexes have become dominant patterns in animal behavior.

Ganglionic Function. Locomotion is under reflex control in annelids, arthropods, and molluscs, but ganglionic reflexes have in no invertebrates been subjected to such complete analysis as have spinal reflexes in mammals. Invertebrate ganglia should provide excellent material for future analysis of excitatory and inhibitory states. Analyses of behavior in terms of ganglionic reflexes are, however, fairly complete.

1. *Annelids.* A number of segments of the ventral nerve cord of an annelid, isolated from the body musculature, can conduct a peristaltic wave to the region beyond. Normally, however, the wave is reinforced by the segmental reflexes.

Collier (74, 75) suspended 20–40 intact segments of earthworms in a saline bath and found no peristalsis if the worms were carefully balanced. If, however, a slight stretch (0.1–1.0 gram on the recording lever) or a tactile stimulus was applied, peristalsis resulted. Tension and touch elicited peristalsis only if the preparation was normally innervated. Moore (207) anesthetized the tactile receptors with $MgCl_2$ and then initiated peristalsis by applying tension.

According to Collier (73, 74) a tension-induced peristaltic reflex could be inhibited by strong tactile stimulation, e.g., by touching the anterior end during a tension wave. Whether the blocking effect of an antiperistaltic wave is due to central inhibition or to refractoriness somewhere in the reflex chain is not clear. Maintained tension set up rhythmic contractions at a maximum frequency of one every two seconds, and the response might continue for many minutes. The frequency increased to a maximum as the applied weight was increased. If the weight was applied for only 0.5 second, a rhythmic response occurred, a sort of after-discharge which might last as long as 3 minutes. A ventral strip containing the nerve cord gave a single contraction, a dorsal strip no contraction, and a piece of intact worm rhythmic contractions in response to stretch (Prosser and Altschul, unpublished).

Gray, Lissmann and Pumphrey (120) made a similar analysis of locomotion in the leech and found that not only tension and contact but also exteroceptive stimuli from the sucker were capable of eliciting peristaltic reflexes. The leech nerve cord showed asynchronous spontaneous electrical activity when isolated, and a rhythm corresponding to the contractile rhythm when connected to an active piece of the body wall. Peristalsis could be started in an earthworm (119) by electrical polarisation with the anterior end positive, and peristalsis could be inhibited by reverse polarisation.

Peristaltic locomotion in the polychaete, Arenicola, is likewise controlled by segmental reflexes, and stimuli at the anterior and posterior ends cause opposite parapodial movements (164). Motion pictures of Nereis in locomotion (118) showed patterns of movement involving clumps of 4–8 parapodia successively. Accompanying the wave of parapodial beat the longitudinal muscles on the same side contracted while those on the opposite side relaxed. Thus the Nereis crawled in the direction in which the wave traveled over the body and not, as in the earthworm, in the opposite direction.

The earthworm peristaltic reflex is segmental or bisegmental in character and involves reciprocal innervation of longitudinal and circular muscles. Circular muscles contract while longitudinal muscles relax and the diameter of the animal is reduced. Then longitudinals contract, shortening the worm. Knowlton and Moore (176) found strychnine sulfate to stimulate the motor system for both sorts of muscle so that under strychnine the worm shortened and also constricted. It appears, therefore, that normally one group of motoneurones is inhibited while the antagonists are excited. Janzen (151) described correlative reflexes controlling the setae. The locomotor reflexes have both homolateral and crossed components.

In general, then, the rhythmic contraction waves associated with peristaltic locomotion in annelids require chain reflexes involving tactile or muscle or sucker receptors and antagonistic central arcs. What recovery mechanisms are so slow as to determine the frequency of contraction under continuous stimulation is not clear but they may well be in the muscle rather than in the central nervous system.

2. *Arthropods.* Arthropod locomotion depends upon central conduction and

upon segmental reflexes which show more complex connections than in annelids. In larvae of the beetle Lucanus cervus Sasse (271) found peristalsis to depend absolutely upon the integrity of the nerve cord. When a segmental nerve was cut that segment was paralysed but conducted a wave to succeeding segments if the cord was intact. Mori (211) observed loss of tonus in skeletal muscles of a silkworm if the 2nd and 3rd thoracic or first abdominal ganglia were removed. In several other lepidopteran larvae (noctuids) each segmental ganglion serves its own segment and the one ahead (145).

The reflex control of insect walking and flight and breathing is very precise. In 1899 Pompilian (235) noted some automatism of thoracic ganglia for leg movements in Dytiscus. Flight can be initiated reflexly by removing the legs from support, i.e., tarsal contact inhibits flight (98). Rijlant (259) recorded potentials from leg muscles of scorpions, crayfish, and some beetles. He found tonic low potential discharges 30–40/sec. during rest and muscle potentials of higher amplitude and frequency during activity. When he isolated one or two segments—ganglion and limbs—reciprocal alternate contractions of flexors and extensors in successive segments occurred. Pringle (237) made similar observations on the cockroach and noted crossed inhibition.

Von Buddenbrock (53) performed a variety of operations upon the nervous system of the walking stick, Dixippus, and concluded that each leg by its motion has a stimulating effect on neighboring legs; excitation passed across a given ganglion and elicited a response from whichever adjacent ganglion was more excitable. Bethe (38) upset the normal sequence of leg movement by removing legs from various spiders, crabs, beetles, etc. Changes in co-ordination occurred at once. Legs which previously alternated now worked together, pedipalps might function in locomotion, swimming was carried out by other legs than normally. Holding a leg was not equivalent to removing it. The changes occurred too fast for learning and must represent pre-existing pathways and an adaptive property of the ganglia.

Ten Cate (302) performed amputations upon the appendages of the cricket, Locusta viridissima, and obtained alterations in co-ordination if he removed a foot only. He concluded that the normal sequence and rhythm is set up reflexly, and is not a pre-established central pattern. Not only can a change in leg rhythm be brought about by amputation but similar changes occur with normal changes in gait. The mantis leg order, for example, (262) when quietly walking is L 3, L 2, R 3, R 2; when excited and walking fast the rhythm is L 2 + R 2, L 3 + R 3; when climbing L 3 + R 1, L 2 + R 3, L 1 + R 2. In some insects the order of leg movement depends much on the surface, i.e., the order is determined by proprioceptive reflexes (146).

Prosser (242) recorded action potentials from various segmental nerves and from the central nerve cord of the crayfish. By stimulating different appendages and making appropriate cuts he obtained a functional map of the nerve cord. The available connections are multiple; which ones are used in a given behavior pattern must depend upon the strength of stimulus and upon the ease of passage through a particular reflex arc.

Locomotor rhythms in insects and crustacea, as in annelids, are reflex in nature and a variety of patterns of locomotion may be used depending upon external requirements.

3. *Molluscs.* In gastropod and pelecypod molluscs, locomotor reflexes are mediated by three pairs of ganglia, cerebral, pedal and visceral.

Pavlov in 1885 (233) experimented with the mussel, Anodonta. Spontaneous activity of the foot stopped when the visceral ganglion was removed but tonus could still be maintained in the adductor muscles after they were denervated. The posterior adductor muscle received motor (exciting) impulses from the visceral ganglion, the anterior adductor from the cerebral ganglion, both adductors received inhibitory fibers from the cerebral ganglion. In the razorshell clam, Ensis (85) local stimulation of the foot failed to elicit general responses if the sensory connections in the cerebral ganglia were eliminated. In Mytilus (331) all of the ganglia act somewhat independently, e.g., if the cerebral ganglion is removed the foot still spins and creeps, due to pedal control; if the visceral ganglion is removed opening and closing reactions to changes in freshness of water are lost. All of these observations argue for fairly restricted control of local areas by specific ganglia in pelecypods.

In gastropods the pedal ganglion gives off a series of nerves which appear to discharge successively to elicit peristalsis along the foot (41). Co-ordination in the large marine gastropod, Aplysia, has been extensively studied. When the pedal ganglion was removed (159, 161) the foot showed more resistance to stretch (tonus) and contractions occurred, particularly at the foot margins. When the cerebral ganglion was removed there was increased locomotor activity and increased excitability of the "wings" (foot). It has been suggested, therefore, that the cerebral ganglion inhibits locomotor functions of the pedal ganglion and that the pedal ganglion inhibits tonic mechanisms in the foot (161). Ten Cate (297) stimulated the central ends of nerves from the "wings" and found that each contains sensory fibers which reflexly through the pedal ganglion elicit contractions in certain areas of the other side of the foot. The cerebral ganglion was not necessary for this response. If the interpedal commissure was cut, only the homolateral responses persisted. In Helix, foot potentials indicated that the cerebral ganglion inhibits motor cells of the pedal ganglion (132).

Ganglionic reflexes control the muscles and chromatophores of the mantle and fin of cephalopods. The mantle nerve goes from the brain mass to the mantle or stellate ganglion and from this the stellar nerves pass to the mantle. Fröhlich (107, 108) found that the mantle ganglia maintained tonus of the muscles and chromatophores of the mantle. Stimulation of the mantle nerve at low intensity inhibited the stellate ganglion, at higher intensities excited it. Ganglionic delay of 10 msec. occurred and summation was indicated. Fatigue of a stellar nerve did not affect the mantle nerve. When the mantle nerve was cut a weak reflex response could be elicited through the stellate ganglion alone. On the contrary Bozler (51) maintained that the stellate ganglion is a motor center which cannot mediate reflexes of the mantle but Ten Cate (299) confirmed Fröhlich and showed that reflex contraction could be elicited over either of two path-

ways, via the brain mass (traversing the stellate ganglion for both afferent and efferent fibers), or via the stellate ganglion alone. Electrical studies on these widely separate ganglia should be possible.

Molluscs, in general, have well developed local reflex centers which may show some interaction but which have relatively restricted control of particular sensory and motor areas. In this respect molluscs are even more highly specialized than the segmental arthropods.

Spontaneous Ganglionic Activity. Locomotion in annelids, arthropods and molluscs is reflex in nature. Isolated portions of the central nervous system in annelids and arthropods show continuous spontaneous electrical activity. Among molluscs such activity has been seen only in the slug, Ariolimax (59). This activity does not in most cases represent summated rhythmic discharges of many neurones as seems very largely to be the case in the vertebrate brain but is asynchronous (240, 244). In most ganglionic chains each neurone is independent of adjacent ones although a given neurone may discharge more or less rhythmically, different neurones having different frequencies. The spontaneous impulses find their way out to body muscles and are tonic in character (152); incoming sensory messages are superimposed upon the spontaneous background.

Certain invertebrate ganglia, however, do show synchronized rhythms. Bullock (59) obtained slow waves upon which spikes were superimposed from central ganglia of grasshopper and Limulus. Breathing rhythms appear to be intrinsic in respiratory centers, at least in many insects. Lesions to specific abdominal ganglia have been shown to stop the breathing rhythm. In Schistocercus, a locust, Fraenkel (99) found breathing centers in abdominal segments 5 and 8, whereas centers for closing the stigmata were in the thorax. In Limulus all or part of the chain of abdominal ganglia, when entirely separated from the rest of the nervous system, can maintain normal movements of the gills (150). Strong support of the neurogenic origin of breathing rhythms in arthropods came with the observation of waves of nerve impulses in isolated nerve cords at rates corresponding to breathing rates (Dytiscus, 1, cockroach 244). These breathing centers are subject to much sensory regulation. In certain insects during flight an increase in breathing amplitude and opening of stigmata and a decrease in breathing rate were found (99).

Another summated rhythm in an arthropod ganglion is the synchronization observed by Adrian (2) in the optic lobes of the brain of Dytiscus. When the eyes were illuminated a rhythm at 20–40 waves per second built up in a synchronization of most of the neurones in the lobe. In darkness this "light" rhythm stopped but after some hours a "dark" rhythm at 7–10 waves/sec. appeared.

Another type of summated rhythm is found in neurogenic hearts. The pacemaker region of the heart of most crustaceans, insects and probably annelids is a ganglion or group of nerve cells (246). As with the asynchronous central ganglion it has been possible to analyse the heart rhythms in terms of single neurone activity. In the Limulus heart ganglion each large pacemaker cell

appears to give rise to a slow wave of negativity for each heart beat and then these and small motor cells nearby discharge a volley of impulses (131, 247). These impulses start at a high frequency and taper off toward the end of the volley.

Other invertebrate ganglia are capable of spontaneous rhythms. The so-called sense organs at the margin of a medusa initiate the pulsations of the jelly-fish (266, 199, 49). Wells (317) described a complicated rhythmic activity of the esophagus of Arenicola, the origin of the wave of nervous activity apparently being in part of the stomatogastric nervous system.

Many invertebrate ganglia, then, show spontaneous activity; this may be asynchronous or synchronized. Single neurone analyses can be made more easily than with vertebrate nerve centers, and some of these ganglionic preparations are useful for elucidating cellular mechanisms of spontaneous rhythms.

Cephalic Dominance. The next step in complexity of nervous function beyond local or segmental reflexes is increasing importance of particular centers near the anterior end of the animal. This importance seems to depend first upon the concentration of sense organs at the front end of the animal and second upon co-ordinative functions of the "brain" ganglia.

1. *Flatworms.* The sensory basis for cephalic dominance is illustrated in the free-living flatworms where the brain is associated with eyes and other sense organs. A number of papers deal with the effects of removal of the brain from flatworms. In the polyclad Thysanozoon the brain is needed for spontaneous locomotion (183), and in the polyclad Yungia immediately after removal of the brain or head the brainless worm or remaining posterior part is inactive and less sensitive than normally but can be stimulated mechanically or chemically to perform swimming movements (208). In Yungia some hours after the operation spontaneous and co-ordinated but undirected movement occurs. Olmsted (216) found in some marine polyclads that swimming stopped but ciliary action persisted if the brain were removed or split. The rippling movement (ataxic locomotion) continued, but extension, placing and release (ditaxic locomotion) and peristalsis stopped since these required conduction from the brain back over lateral cords. Righting appeared to require the brain in the polyclad Planocera (209), but not in the triclad Planaria (183). The greater importance of the brain in polyclads than in triclads may be associated with the distribution of ganglion cells. In Planaria locomotion and reflex extension of the pharynx and swallowing continue after removal of the head but normal behavior to food reappears only after the brain regenerates (23).

In cestodes after the lateral cords are cut a wave of contraction progresses backward across the cut, being conducted reflexly by tension applied behind the cut (254). Friedrich (103) found that cutting one lateral cord resulted in circus movements in those species of nemerteans in which transverse connections between lateral cords were incomplete, but not where the cords were connected. In summary, the flatworm brain relays sensory messages and co-ordinates motor responses, there being no other ganglia; its directive function is, therefore, very important.

2. *Annelids.* An earthworm has sensory cells of various types—tactile, chemical and light receptors, scattered throughout the epidermis, but most concentrated in the prostomium and anterior segments. Only the sense cells which are sensitive to dryness seem restricted to the prostomium (232). Nereis, on the other hand, has fewer epidermal receptors; its effective chemoreceptors are on palps and tentacles and instead of scattered photosensitive cells it has several pairs of eyes on its head. After the brain is removed from an earthworm the anterior segments are lifted upward, it crawls normally, appears restless and active, it can right itself, can copulate, eat, and burrows in half an hour as compared to a normal time of one to two minutes (198, 239). After the brain is removed from a Nereis it no longer feeds, does not burrow, is over-active and has lost its light sensitivity and most of its chemical sensitivity (183, 198). When the sub-esophageal ganglion is removed from either species there is no burrowing and the worms are very quiet. The brain, then, is a sensory center and it normally has an inhibitory or restraining control over the motor centers in the subesophageal ganglion.

An example of integrative cephalic dominance which has a simple morphological basis is found in the responses of an earthworm to light. An earthworm normally responds negatively to lateral illumination. When the brain is removed the direction of its response is reversed (134). If the ventral cord is cut a few segments behind the subesophageal ganglion the anterior tip turns away from the light while the region behind the cut tends to bend toward the light. If one esophageal commissure is cut the worm tends to circus toward the normal side and when illuminated from the intact side the worm turns toward the light but when illuminated from the operated side turns away from the light (238, 148). Apparently the scattered photoreceptors set up a homolateral reflex in the ventral ganglia, a positive response to light; the tracts from the prostomium and first two segments, however, cross in the brain and cause a negative response. The brain normally dominates but at very low light intensities a positive response may occur.

The brains of some polychaete worms are complex and have lobes corresponding to the specialized forebrain centers of arthropods (123). These lobes (corpora pedunculata) are best developed in free-swimming polychaetes which have cuticular eyes; they do not occur among oligochaetes. It has been shown that Nereis (77) can be taught simple associations; Lumbricus (334, 130) and Lumbriculus (252a) can be taught even when the brain is not present. An investigation of integrative functions of the brain of Nereis should be very interesting in view of the complexity of its brain.

3. *Arthropods.* There is much variation in complexity of the brain among different groups of arthropods. In general there are three regions: the protocerebrum, the deutocerebrum and the tritocerebrum. The bulk of the lateral portions of the protocerebrum is devoted to vision centers which are directly connected to the eyes; the middle and anterior portions contain the association areas, the protocerebral bridge, the central body, and the large cellular corpora pedunculata. There are no corpora pedunculata among some lower crustacea. The

deutocerebrum usually lies ventro-anteriorly and may contain large antennal centers as well as "olfactory" lobes. The tritocerebrum lies behind and customarily gives rise to certain nerves going to mouthparts as well as to the stomatogastric nerves; the tritocerebrum is continuous with the circumesophageal commissures.

In view of the complex instinctive behavior and limited but real modifiability of behavior in arthropods, especially among insects, an understanding of the function of different parts of the brain should be useful. Hanström (123) has compared the relative portions of the brain occupied by different regions in various groups as follows:

Vision centers	Arachnoidea and Myriapoda	epithelial photoreceptors and ocelli	0.3- 2.8%
Vision centers	Crustacea and Insecta	rudimentary compound eyes	2.9- 9.9%
Vision centers	Crustacea and Insecta	higher compound eyes	33.0-80.0%
Olfactory centers	Crustacea		10.0-30.0%
Olfactory centers	day-flying insects		1.3- 4.4%
Olfactory centers	one night-flying insect		13.4%
Olfactory centers	worker ant (Formica)		18.1%
Central body	Arachnoidea		1.6-15%
Central body	Crustacea		0.2- 0.5%
Central body	Insecta		0.2- 0.6%
Corpora pedunculata	Limulus		78.7%
Corpora pedunculata	Arachnoidea		2.0-49.0%
Corpora pedunculata	decapod Crustacea		10.0-30.0%
Corpora pedunculata	isopod Crustacea		0.0%
Corpora pedunculata	Odonata, Lepidoptera, Diptera		2.0- 9.6%
Corpora pedunculata	worker ant (Formica)		40.4%

Since the days of Aristotle, the observation of behavior of arthropods from which parts of the body have been removed has been a favorite pastime. A complete survey of papers up to the year 1930 dealing with the effects of operations on the nervous system is given by Ten Cate (301).

The following is a list of the most important animals on which operative studies on locomotion have been made, with references to the papers.

Crustacea. Squilla—35; Palaemon—82, 86; Homarus—196; Astacus—35, 124, 160, 287, 314; various crabs—34, 82, 86, 133, 234, 287, 312; Cancer—160; Maja—287; Eupagurus 300; Portunus—133; Oniscus—287.

Xiphosura and *Arachnida.* Limulus—150; various spiders—260.

Myriapoda. Lithobius—67, 153, 287; Julus—70, 287; Scolopendra—62.

Insecta. Dytiscus—96, 235; Hydrophilus—35; Carabus—19, 111, 296; Lucanus larvae—271; Melanoplus—95; Mantis—262; Ranatra—140; Periplaneta—20, 21, 296; dragon fly nymphs (Aeschna larvae)—22, 313, (Cloeon and Agrion larvae)—3; Apis—35; Lymantria larva—177.

Similarity of results indicates that further investigations by these gross methods would not be fruitful.

Removal of any part of the brain which receives tracts from any sense organ is equivalent to removing that sense organ. Immediately after removing a sense organ or injuring the brain, there may be a shock reaction of general incoordination. Thereafter, specific behavioral deficiencies appear. Most arthropods can eventually compensate for the removal of one eye or of an antenna, whereas injury to the brain leaves a more permanent behavior deficiency. If both circumpharyngeal commissures are transected, reflexes of the head such as antennary movements remain; otherwise the effect resembles that of total brain removal. Spontaneous directed locomotion ceases, although locomotion is possible under stimulation. Locomotion is less impaired by brain removal in some species (Carcinus and Eupagurus) than in others (Astacus). Mouth-parts may move, but usually there is no co-ordinated feeding. Some animals can chew but cannot swallow after brain removal. Righting and leg reflexes are unimpaired. Usually there is extensive random activity of legs.

When one circumesophageal commissure is cut, or one lateral half of the brain destroyed, the arthropod circuses toward the intact side. Legs on the operated side tend to be flexed and low in tonus so that the animal leans toward this side.

There are many reports that removal of the subesophageal ganglion, or cutting behind this ganglion stops spontaneous activity. Local segmental reflexes, however, persist. The subesophageal ganglion is apparently the principal motor center, but the other thoracic and abdominal ganglia are capable of carrying out reflexes of locomotion, autotomy, and so forth; these are normally regulated by the subesophageal ganglion.

The increased leg activity after brain removal suggests the release of the ventral motor centers from inhibition by the brain. Similarly, when the brain was removed from dragon-fly larvae (197, 84), there was an increase in breathing frequency (although recovery of the normal rhythm has been reported (313)), while when the subesophageal ganglion was removed, the breathing frequency (determined by posterior ganglia), was decreased. Death-feinting is a function of the nervous system as a whole rather than of any particular ganglion; in some insects and in spiders it is relatively unaffected by brain removal. In others, as the spider Celaenia (260), there is no death-feinting after brain removal. In Ranatra, the death-feinting reaction is shortened after brain removal (140); the posterior half of the body with nerve-cord cut comes out of a feint sooner than the part of the body containing the brain. The most convincing evidence for inhibitory action of the brain on the ventral ganglia comes from electrical stimulation. Jordan (160) stopped circus movement in a crab by a weak electrical stimulation of a transected circumesophageal commissure. Celesia (65) caused a flexion of the abdomen of Palinurus and Homarus by tetanic stimulation of the abdominal nerve-cord; if during this stimulation the brain was also stimulated, the abdominal response was diminished. Caselli (64), also working on Palinurus, stopped the responses of tail and cloaca to abdominal stimulation by simultaneous stimulation of the circumesophageal commissures.

The interaction between the brain and subesophageal ganglia is clearly shown by the work of Roeder on the praying mantis (262). When one side of the protocerebrum was removed the legs lost tone on the operated side, the legs on the operated side were more active and the leg sequence changed, circusing was to the normal side. When both protocerebral lobes were destroyed, leg tonus was lost on both sides, there was locomotor restlessness, the mantis walked straight ahead, did not avoid objects, and showed no head motility or backward walking. When the protocerebrum was split there was decreased leg movement, high neck and prothoracic tonus and active visual responses (following moving objects). When the subesophageal ganglion was removed there was no locomotion except to strong stimulation. Roeder concluded that the subesophageal ganglion excites locomotor activity in thoracic ganglia, that the protocerebral ganglion has inhibitory centers controlling the irritability of the subesophageal locomotor center and excitatory centers maintaining tonus and activity in neck and prothoracic muscles; these protocerebral centers are homolateral, are inhibited contralaterally by each other and strengthened by homolateral sensory impulses. Roeder noted that sometimes in mating, after clasping, the female eats the male, head first, and copulation continues actively. Experimentally he found (261) that if the brain was removed there was no sexual activity, but if the subesophageal ganglion was removed the male made copulatory movements and the female would receive the male. The copulatory center is in the last abdominal ganglion and this is normally inhibited by the subesophageal ganglion. Thus the eating of the head (and subesophageal ganglion) by the female actually releases copulatory activity!

Some arthropods exhibit very complex behavior, some of which may require the presence of the brain. A brainless hermit crab for example (300) will not re-enter its shell unless it is helped by having the telson pushed in; the hermit crab will grasp a new shell but fails to investigate it as a possible dwelling-place. In some arthropods (female Bombyx—201, and Carcinus—35), mating can occur in brainless individuals, whereas in others (various butterflies,—177), it does not. There is ample evidence that some insects have capacity for learning. Von Frisch (105), for example, taught honey-bees to come to glass dishes set over certain colors and patterns. Experiments concerning the possible relation of parts of the brain to learning should be very profitable.

The function of the specific lobes of the brain can be partly inferred from their connections as shown in the table from Hanström given above. Bethe (36) found that removal of the globuli (the cellular portion of the corpora pedunculata) in Carcinus caused the loss of certain responses to light, and of sidewise locomotion; he stated that the globuli are inhibitory centers of the brain. In the myriapod Lithobius (153) the protocerebrum inhibits the ventral motor centers and controls the sense of direction. Roeder's results are in agreement (262). Ewing (95) on the other hand, stated that the legs of a grasshopper went into constant motion when only the posterior part of the brain was removed.

In summary, then, the brains of arthropods not only are connected with the most important sense organs of the body, but they also exert important in-

tegrative, particularly inhibitory, control of the ventral motor centers. It should be profitable to investigate further localization of function within the arthropod brain and the mechanisms of interaction between the brain and ventral ganglia, and between the subesophageal and other ventral ganglia.

4. *Cephalopods.* Cephalic dominance has developed in a somewhat different manner in cephalopod molluscs. The brain and some thoracic ganglia of arthropods represent fusion of several ganglia. The cephalopod brain-mass represents fusion of paired cerebral, pedal, and visceral ganglia, and completely surrounds the esophagus (309, 270). Paul Bert in 1867 (33) was the first to show that much of the supraesophageal portion of the brain-mass was, like the association areas in the mammalian cerebral cortex, electrically inexcitable with respect to behavior. He also showed that a respiratory center exists in the subesophageal portion of the brain-mass. Von Uexküll (309) divided this respiratory center into regions for inspiration and expiration. He and other early workers (172) also found a center in the subesophageal portion which regulates chromatophores. The subesophageal portion contains other local centers (270): the brachial and pedal ganglia controlling arms and tentacles, the pedal ganglion controlling funnel and eye-muscles, the pallio-visceral ganglion controlling mantle, fins and viscera. In Octopus a center for pupillary closure is found in the subesophageal portion (316).

The supraesophageal portion (270) contains: (1) Higher motor centers in the circumesophageal region—lobus basalis anterior, posterior and lateralis, lobus pedunculi; stimulation of these lobes elicits movements of large groups of muscles, and unilateral extirpation results in circus movements which may be almost continuous. (2) Primary sensory centers such as the olfactory lobes and the optic lobes in the optic stalk; stimulation of the latter may result in chromatophore expansion and some mantle and fin movement. (3) The dorsally located verticalis complex of three lobes, electrical stimulation of which results in no motor responses. After removal of the entire verticalis complex Sepia can see, swim, steer properly, capture and eat prawns, etc. However, when a prawn which it was hunting went around a corner the operated Sepia failed to follow it out of sight as normal animals did (270). Removal of part of the verticalis complex from Octopus hampered its ability to escape from a cage and from a pan of shallow water (61). By conditioning experiments specimens of Octopus (304) were shown to be able to discriminate between square and circle while Sepia (270) was trained not to eject its tentacles at a prawn behind a glass bearing a white circle but to continue to shoot them at prawns not accompanied by glass and circle. Sanders and Young (270) conditioned three individuals not to eject their tentacles at a prawn behind a glass plate; "memory" of this lasted eighteen hours; the learning of this association occurred after about the same number of trials with or without the verticalis complex.

It appears that the verticalis complex is an association area but that conditioning of other centers can occur.

Neuromuscular Integration. Among vertebrates integrative functions are

largely a property of the central nervous system, gradation in control of movement is carried out largely in the ventral columns of the spinal cord. Among most invertebrates there are numerous ganglia each of which can regulate locomotor reflexes. Among many invertebrates much control of gradation of movement is at neuromuscular junctions rather than centrally. A crab "thinks in it claws."

Early histological studies (40, 139, 191) showed: (1) that whole muscles in many crustaceans and insects are innervated by only two (or a few) axons and (2) that each muscle fiber has multiple innervation, i.e., each muscle fiber receives branches from two or more nerve fibers, often from all the motor fibers to the muscle. Triple innervation of claw muscles in the crayfish was described by Van Harreveld and Wiersma (307) and even quintuple innervation of a muscle of the leg of Panulirus (308). Pringle (236) described double innervation of leg muscles of a cockroach. It is likely that multiple innervation of fibers and innervation of all the fibers of a muscle by the same axon is a general rule among higher crustacea and insects.

Stimulation of claw nerves of Crustacea early revealed that at low intensities of repetitive stimuli the adductor muscle contracted while at higher intensities the same muscle relaxed (was inhibited) and the abductor contracted (40, 106, 139, 311). It appeared that the peripheral inhibition was associated with double innervation. Keith Lucas (184, 185) showed that in the claws of lobsters and crawfish a slow low-level contraction of the adductor resulted from a weak, long-duration stimulus and a rapid strong twitch from a strong brief shock. On the basis of thresholds, effective stimulus durations and separate intensity-duration curves he concluded that there were two *excitatory* systems to the same muscle, one fast and one slow. Paired stimuli resulted in summation of response in each system.

A further complication in crustacean neuromuscular conduction is the fact that sometimes single strong shocks applied to a crustacean nerve elicit repetitive discharges in the nerve (24). This repetitive response depends upon the calcium in the medium bathing the nerve (166). Barnes suggested (24) that gradation in response depends on the frequency and duration of the repetitive discharge. This might explain the following observations of Blaschko, Cattell and Kahn (43) on the claws of Homarus, Cancer, and Maia. The tension of the contraction increased gradually as the frequency of repetitive stimuli increased, more muscle fibers coming in with increasing frequency. Normally the slow closing response occurred at 2 to 10 per sec. and the fast response at 50 to 100/sec. If the slow type response was being elicited and a single extra or strong shock interposed, suddenly the contraction changed to the fast closing type and continued at this level even though stimulation was at the original low level.

Crustacean neuromuscular junctions, then, behave very differently from those of vertebrates. Segaar (277) suggested that the effect of a nerve depends on the tonic state of the muscle so that some fibers can evoke either excitation or inhibition. This suggestion lacks experimental verification. Segaar also claimed that stimulation of the brain elicits opposite responses from stimulation of a

peripheral nerve (133, 277). Tonner (305) claimed to find a subepidermal network which maintained tonus in the muscles and which was influenced by impulses from the nerve centers. His views have found no support.

Pantin in a series of papers (218, 219, 224, 225, 226) analysed the effects of frequency, duration and intensity of stimuli to the nerve upon responses of walking leg muscles (largely flexors) in Maia and Carcinus. At low intensities there was little or no response to a single shock, a greater response after several shocks. The response (tetanus) increased with increasing frequency or increasing intensity. With intensities just above threshold most units were brought into action at 60–100 shocks per second and a maximum response occurred at 300 per second. Thus the response depended upon the number of impulses arriving in the muscle in a given time. If the frequency or intensity were increased abruptly by 10–20 times threshold the rate and height of contraction increased, i.e., a quick response was brought in. This Blaschko, Cattell and Kahn effect could also be obtained by stretching the muscle during repetitive low intensity stimulation. When the quick response was fatigued the slow response was also. Pantin contended that the bringing in of the quick response was due to repetitive nerve impulses set up by the interposed stronger stimulus. This would make for economy as a few extra impulses coming from the nervous system on a low frequency background could change the response from the slow to the fast type. If brief tetani were given at increasing intensities and both the flexor (adductor) and extensor (abductor) muscles studied there was first a contraction of the extensor, then at a higher intensity the threshold of the flexor was reached but the extensor contraction diminished and at still higher intensities the flexor was inhibited. Thus Pantin arranged the four fibers in order of increasing threshold as follows: excitor of extensor, inhibitor of extensor, and excitor of flexor and finally inhibitor of flexor. He also was able to separate the four fibers by strength-duration curves. The inhibition was less effective against high frequency stimulation (fast contraction) than against low frequency stimulation. Pantin concluded that the response in the crustacean neuromuscular system of the walking leg depends upon facilitation, more muscle fibers responding as more impulses arrive at the muscle, that the fast response is the result of high frequency impulses, that the Blaschko, Cattell and Kahn effect is due to repetitive firing and that inhibition results from impulses in the second or higher threshold axon innervating a muscle. In the crusher claw Pantin obtained evidence for a double excitor system controlling the slow and fast responses. Pantin's views were supported by Katz (166) who demonstrated summation of both electrical and mechanical responses of the Carcinus walking leg. He found the largest increase in number of responding fibers at between 60 and 120 shocks per second and noted repetitive nerve responses to a constant current.

Electrical responses have frequently been recorded in crustacean muscles. DuBuy (87) used tiny knob electrodes for recording and detected a fast nerve potential followed by a slower muscle response. He found a negative excitatory wave and a positive inhibitory wave and stated that the muscle would respond to adrenalin without an action potential. The observation of positivity in

inhibition has not been repeated. Serkoff (278) stated that inhibitory impulses to a resting muscle did not alone set up any electrical phenomena but might or might not reduce the height of the potential in a contracting muscle. Sommer (286) supported Pantin's views that the response is a function of the frequency of stimulation; in Astacus he found that an increase in frequency by ten times increased the amplitude of the potential about three times; stimulation of the inhibitor nerve and of the excitor simultaneously diminished the muscle potential while stimulation of the inhibitor nerve alone resulted in no electrical wave in the muscle.

From 1933 to the present Wiersma and his collaborators, particularly Van Harreveld, have published a series of papers which constitute the most extensive and definitive work on this subject. Wiersma's chief technical contribution is the isolation and identification of function of single motor nerve fibers. It had been indicated earlier and definitely shown by Knowlton and Campbell (174) (also Knowlton, 175) that the claw nerve of the lobster could be split into bundles. One containing the larger motor fiber caused contraction of the adductor and inhibition of the abductor. The smaller fibers on the other hand had the reverse effect. Wiersma and his associates have traced the course of individual fibers by methylene blue staining and have identified their action by stimulating the separate nerve fibers and recording contractions and muscle action potentials. Their work was summarized at length in 1941 (321) and only a brief description of it can be given here.

The variation in pattern of nerve fiber distribution among different species and in different muscles of one species is remarkable. Some muscles receive one excitatory and one inhibitory fiber; in these muscles the height of contraction depends upon facilitation; the walking leg muscles studied by Pantin are examples. Some muscles receive two excitatory and one inhibitory fiber. In these the largest fiber, the "fast" one, causes a contraction of short latency, rapid shortening, and high tension development; the fast fiber may elicit an action potential which appears with the first of a train of impulses and shows slight facilitation for the first few spikes after which the contraction begins (306). Stimulation of the second or intermediate fiber, the "slow" one, causes a contraction which builds up very slowly so that there is an apparent long latency and a low tension; the muscle action potentials are smaller than from the "fast" fiber stimulation, they may not appear until after several stimuli and they show much facilitation. Stimulation of the third and smallest fiber causes inhibition of contraction, reduces the height of contraction and in some muscles reduces the height of the action potential; inhibition is more effective against the slow than against the fast contraction. During complete inhibition no contraction occurs, but facilitation continues to be built up in the slow system as shown on release from inhibition. The distinction between "fast" and "slow" responses is quantitative rather than qualitative. At low frequencies (40–50/sec.) the difference between the two fibers may be very slight or the larger fiber may even give a smaller contraction but at high frequencies (over 100/sec.) the fast contraction is much greater (326). Thus the optimum frequency in the slow fibers is lower

than in the fast fibers. The difference between the fast and slow contraction of the same muscle becomes less in the species series Cambarus > Randallia = Blepharipoda > Cancer so that in Cancer there is little difference between the two except latency (326). The flexor of the carpopodite of Panulirus has five axons, 4 excitors of different rates and one inhibitor (308). The amount of facilitation required to elicit a contraction varies with the species, e.g., in the crayfish the fast system gives a detectable response to one shock, the slow system requires more than one while in Blepharipoda repetitive stimuli are required for both systems (327). The ratio of optimum inhibitory to excitatory frequencies is fairly constant for any given system (323). For example, in the slow bender of Pachygrapsus three excitor impulses are suppressed by one inhibitor, in the slow closer of Cambarus one excitor requires three inhibitor impulses, while the fast closers of these species respond to single excitor impulses and are uninhibitable.

In several instances one nerve axon serves two muscles (320). For example in the crayfish claw (Cambarus clarkii) the dactylopodite (pincer) abductor has one excitor which it shares with the extensor of the protopodite while the latter muscle shares its inhibitor with the adductor of the dactylopodite (307, 329). In Cancer (322, 324) the abductor of the claw has two inhibitor fibers, one its "true" inhibitor reduces muscle potentials if stimulated just before the excitor, the other its "common" inhibitor is shared with the adductor of the same segment and is without effect on the potential of the abductor but reduces its contraction and slightly reduces the potential of both fast and slow systems of the adductor. Often reciprocal innervation does not apply for a pair of antagonists and at least one muscle receives two inhibitors (322). Regenerated claws may have more motor axons than normal claws (306, 25, 320).

When both excitor and inhibitor fibers are being stimulated repetitively, the inhibitor fiber may cause diminution of both contraction and action potential if its impulses arrive less than 10 msec. before the excitor impulses ("supplementary" inhibition), or the inhibitor may cause reduction of contraction only, if its impulses precede the excitor impulses by longer times ("simple" inhibition) (191a).

All evidence indicates that every fiber in a muscle receives a branch from every axon. Each fiber, then, must contain several excitation systems. Keighley and Wiersma (167) showed that the slow system is more efficient than the fast one in that its ratio of heat to mechanical work is lower. However, chemical changes are of the same order with the two types of contraction (32). It is possible to produce fatigue and facilitation among the different excitor systems separately (328); one inhibitor can suppress two or more contraction types. How so many types of response can be elicited in the same muscle fiber is a major problem for muscle physiology. Wiersma (321) presented the following scheme for each excitor system: nerve impulse → transmission process → muscle potential → transmission process → contraction. He suggested that facilitation can occur at either transmission process and that the contraction system is common to several kinds of nerve impulse; "simple" inhibition blocks between

the muscle potential and the contraction, whereas "supplementary" inhibition blocks between the nerve impulse and the muscle potential.

In general, crustacean muscle differs from vertebrate striated muscle in showing multiple innervation; the same axon serves many muscle fibers so that conduction throughout the muscle can be nervous; some muscle fibers receive axons which elicit contractions of different speeds and tensions; facilitation is essential for most contractions, more for the slow than the fast ones; a nerve fiber may discharge repetitively to a single stimulus; there are specific inhibitory nerve fibers. Regulation of the height and speed of a contraction is brought about by (1) variation in frequency (facilitation), (2) action of fast and slow nerve fibers, (3) inhibitor nerve fibers, and (4) by continuation of a high level response at low level stimulation after a few high frequency impulses.

Multiple innervation of muscles exists not only in Crustacea but also in insects (191). Each of the leg muscles of a cockroach receives two nerve fibers (237). One of these causes a tonic contraction and discharges when attached normally to the nervous system at 30/sec. while the other gives bursts of large spikes at 75/sec. and causes a quick high-level contraction (236). The slow system shows much facilitation, the fast system little or none. No inhibitory fibers were found, but antagonism between extensors and flexors involving central inhibition in unisegmental reflexes was shown by appropriate sensory stimulation of intact preparations (237). Friedrich (104) obtained functional evidence of multiple innervation in the control of peripheral inhibition of the leg muscles in Dixippus.

In the clam Mya arenaria, the adductor muscles and mantle retractor muscles receive two types of nerve fiber (249). One fast type elicits a rapid contraction with large action potential, the other type causes a prolonged tonic type of contraction with a low level electrical response. Such a system is efficient when a low level contraction must be maintained and occasional quick contractions superimposed. The retractor of the buccal mass of Helix appears not to be doubly innervated (253). In Pecten the adductor muscle is composed of a striated portion used for rapid swimming movements and a smooth portion used for prolonged maintained contraction (26). During swimming the smooth muscle is inhibited and part of this inhibition is probably peripheral (30).

We have found no information as to whether multiple innervation exists among annelids or echinoderms.

Many invertebrates, then, carry out much regulation of movement by peripheral neuromuscular mechanisms, regulation which in the vertebrates is carried out in the spinal cord.

Chemical Agents. The nature of synaptic transmission in invertebrates is virtually unknown. No analyses of ganglionic potentials have been made although many measurements of synaptic delay are available.

In view of the importance of acetylcholine in sympathetic ganglia, at certain neuromuscular junctions and at numerous other nerve endings in vertebrates it is reasonable to inquire into the origin of cholinergic systems. The effects of acetylcholine and adrenalin upon invertebrate hearts, chromatophores, and

visceral musculature are not pertinent to this review. It should be said, however, that evidence from a few perfusion experiments and from the effects of drugs upon invertebrate hearts indicates that acetylcholine may have much to do with cardiac regulation in many animals. Acetylcholine accelerates neurogenic hearts (most arthropods and probably most annelids) and inhibits myogenic hearts (molluscs and vertebrates) (246).

Information concerning cholinergic systems can be obtained from (1) measurements of cholinesterase content of nervous and muscular tissues, (2) measurements of acetylcholine (Ach) in these tissues, and (3) sensitivity to acetylcholine and to its potentiating and antagonizing drugs. Available data on cholinesterase content of invertebrate tissues and a few representative vertebrate tissues are summarized in table 3. The data presented in this table are not all comparable because of differences in method and in laboratory. The highest concentrations of cholinesterase reported in vertebrate tissues are in electric organs of fish, the highest in mammalian nervous tissue are in sympathetic ganglia (except dog lenticular nucleus). A ganglion of the squid is the highest invertebrate tissue reported although the lobster ganglia, honeybee brain and Limulus heart ganglion appear to be rich in cholinesterase. Cholinesterase is found in nervous and muscular tissues in all of the groups tested down to coelenterates and ctenophores. Among coelenterates Bullock and Nachmansohn (60) found the enzyme in the hydrozoans and anthozoans Tubularia, Metridium and Sagartia but found none of it in any regions of the medusae Cyanea and Aurelia; they found none in the ctenophore Mnemiopsis. They also found none in the sponge Grantia or in Paramecium although adequate masses of tissue were used. Bacq (10) also found none in Rhizostoma and sponges although he found much in the muscle of Holothuria. The concentration in flatworm mixed muscle and nervous and other tissue is strikingly high.

The activity of invertebrate extracts towards other substrates than ACh has been examined in the honeybee brain where there appeared to be proportionately very much more "true" than "pseudo"-cholinesterase than in mammalian sympathetic ganglia.

Determinations of acetylcholine content are less dependable than determinations of the esterase because of hydrolysis and necessity for quick inactivation of the esterase before assay. Further, there is uncertainty as to how acetylcholine may be "bound" in tissues. Such results as are available are given in table 4. Crustacean ganglia compare favorably with mammalian sympathetic ganglia and some insect ganglia appear to be even higher in acetylcholine content. Octopus ganglia contain the highest concentration yet observed (except possibly for some insects). Bacq and Mazza (17) purified and chemically identified this acetylcholine from Octopus.

The correlation of esterase and of acetylcholine content with responses to acetylcholine is confusing and raises a question of the significance of esterase and acetylcholine, which occur in many groups. Good evidence that acetylcholine plays a part in neuromuscular transmission exists for annelids. In the earthworm and leech the body wall is stimulated to contract by acetyl-

TABLE 3

Cholinesterase in representative nervous and neuromuscular tissues

ANIMAL TISSUE	REFERENCE	Q Ch E mgm. Ach HYDROLYZED BY 100 MGM. TISSUE PER HOUR AT NTP
Vertebrates		
Dog—brain cortex	(212)	2–3
Rat—brain cortex	(252)	4.7*
Guinea pig—brain cortex	(252)	4.6*
Guinea pig—brain cortex	(192)	1.3
Ox—brain cortex	(215a)	1.5*
Ox—caudate nucleus	(215a)	5.9*
Dog—lenticular nucleus	(212)	70.0
Dog—cer. sympathetic ganglion	(212)	14.0
Cat—cer. sympathetic ganglion	(116)	36.0
Cat—cer. sympathetic ganglion	(272)	28.4
Guinea pig—cer. sympathetic ganglion	(272)	12.8
Cat—sympathetic fibers	(272)	3.7
Dog—sympathetic fibers	(212)	5.0
Cat—sciatic nerve	(272)	0.7
Frog—sciatic nerve	(193)	0.78–1.0
Chick (9 day incubation) muscle	(213)	3.0
Chick (20 day incubation) muscle	(213)	9.4
Chick (21 day after hatching) muscle	(213)	0.9
Chick adult muscle	(213)	0.4
Frog—thigh muscle	(192)	0.75
Frog—sartorius (pelvic end)	(195, 193)	0.13
Frog—sartorius (tibial end)	(195, 193)	0.4–0.8
Lizard—muscle	(195)	1.6–3.4
Gymnotus—electric organ	(215)	90–150
Torpedo—electric organ	(215)	150–300
Raja—electric organ	(215)	3–10
Electrophorus—electric organ, anterior end	(214)	400–500
Electrophorus—electric organ, posterior end	(214)	100
Arthopods		
Homarus—abdominal chain	(215a)	2.3*
Homarus—central ganglia	(212, 194)	15–25
Limulus—central ganglia	(284)	6.9*
Melanoplus—brain	(202)	0.408
Honeybee—brain	(257)	16.5*
Astacus—nerve cord	(163)	++
Periplaneta—nerve cord	(203)	0.12
Melanoplus—nerve cord	(202)	0.356
Homarus—nerve fibers	(212)	10.0
Limulus—cardiac ganglion	(284)	14.27
Limulus—whole heart	(284)	1.6
Callinectes—whole heart	(284)	1.63
Pagurus—whole heart	(284)	1.57
Libinia—whole heart	(284)	1.18
Melanoplus—whole heart	(202)	0.04
Melanoplus—thoracic muscle	(202)	0.134
Melanoplus—femur muscle	(202)	0.068
Limulus—skeletal muscle	(284)	0.144
Homarus—skeletal muscle	(195)	0.2–0.5

TABLE 3—*Continued*

ANIMAL TISSUE	REFERENCE	Q Ch E mgm. Ach HYDROLYZED BY 100 MGM. TISSUE PER HOUR AT NTP
Arthopods—*Continued*		
Homarus—skeletal muscle	(18)	0.077
Melanoplus—blood	(202)	0.059
Limulus—blood (serum)	(284)	1.34*
Rat—blood	(252)	1.37*
Guinea pig—blood	(252)	3.51*
Molluscs		
Modiolus—heart	(284)	8.73
Venus—heart	(284)	0.176
Eusepia—muscle	(18)	0.076 avg.
Eusepia—brain	(18)	1.18
Loligo—giant axon (whole)	(44)	0.15
Loligo—giant axon (sheath)	(44)	0.42
Loligo—unspecified ganglion	(215a)	150.23*
Loligo—mantle nerve	(215a)	0.70*
Annelids		
Pontobdella—skin-muscle	(18)	0.047 avg.
Echinoderms		
Asterias—radial nerve cord	(60)	0.384
Thione—radial bands	(60)	0.820
Holothuria—muscle	(18)	0.0745 avg.
Nemerteans		
Cerebratulus—dorsal muscle	(281)	6.86*
Flatworms		
Prototyla—body (Dendrocoelum)	(60)	9.3
Planaria—body	(60)	++++
Ctenophores		
Mnemiopsis—comb plates, etc.	(60)	0
Coelenterates		
Tubularia—heads	(60)	0.267
Tubularia—cleaned stalks	(60)	0.312
Metridium—whole	(60)	0.034
Metridium—oral disc	(60)	0.009
Metridium—muscle	(18)	0.002 avg.
Aurelia and Cyanea—epithelial sheet and related tissues	(60)	2 positive out of 12 preparations
Sponges		
Scypha—whole	(60)	0
Protozoa		
Paramecium	(60)	0

* QChE calculated from data given in reference.

TABLE 4

Acetylcholine content of selected tissues

ANIMAL TISSUE	REFERENCE	Ach CONTENT IN γ/gm.
Vertebrates		
Dog—brain	(179)	1.5-3.8
Dog—brain	(66)	0.4
Cat—brain	(179)	0.4-2
Cat—basal ganglia	(83)	0.4
Cat—sympathetic ganglia (normal)	(52)	10-20
Cat—sympathetic ganglia (denervated)	(52)	1-3
Cat—sympathetic ganglia (normal)	(189)	25
Cat—sympathetic ganglia (denervated)	(189)	7
Horse—sympathetic nerve	(66)	1.8-2.0
Rabbit—outer intestinal layer (with myenteric plexus)	(318a)	10-16
Guinea pig—outer intestinal layer (with myenteric plexus)	(318a)	16-20
Arthropods		
Crayfish—nervous system	(163)	20
Crayfish—ventral nerve cord	(279)	9.8
Crayfish—nerves	(285)	3-12
Crayfish—ganglia	(285)	14-46
Crayfish—blood	(285)	0.7-1.1
Homarus—nerve cord	(279)	15.9
Cancer—nerve cord	(279)	3.1
Callinectes—nerve cord	(279)	6.6
Carcinus—nerve cord	(163)	2-4
Palinurus—abdominal muscle	(9)	<0.1
Cockroach—nerve cord	(203)	70
Insects (series of genera)—ganglia	(78)	35-200
Insects (series of genera)—head	(78)	2.5-50
Insects (series of genera)—blood	(78)	0
Molluscs		
Helix, Unio, Anodonta—nerves and ganglia	(5)	++
Aplysia—esoph. ganglion	(10)	2-3
Aplysia—foot	(10)	0.3
Aplysia—stomach	(10)	0.6
Pectunculus—body	(10)	0.65
Octopus—cerebral ganglion	(10)	77
Octopus—stomach and esophagus	(10)	1.4
Octopus—ovary	(10)	0.2
Octopus—testis	(10)	0
Octopus—mantle muscle	(10)	0.7
Octopus—blood	(10)	0
Annelids		
Spirographis—body	(10)	0.5-0.7
Halla—body	(10)	0.8
Sipunculus—body	(10)	0.8-0.9
Sipunculus—muscle	(10)	0.7

TABLE 4—*Continued*

ANIMAL TISSUE	REFERENCE	Ach CONTENT IN γ/gm.
Ascidians		
Ciona—body	(10)	0
Phallusia—body	(10)	0
Echinoderms		
Holothuria—longitudinal muscle	(10)	1.5-1.7
Holothuria—intestine	(10)	0.4
Coelenterates		
Rhizostoma—body	(10)	0
Alcyonum—body	(10)	0
Porifera		
Leuconia—body	(10)	0
Siphonocula—body	(10)	0

choline and sensitivity is increased by eserine. The action of acetylcholine upon the earthworm body wall is abolished by nicotine (333). Summation of contractile response of the earthworm body wall to repetitive electrical stimulation is increased by eserine (47). Bacq and Coppée (16) found that eserine increased the muscular responses of Sipunculus, Hirudo, Arenicola, Aphrodite and Lumbricus to stimulation of the nerve cord. They found no such effect when the eserine was applied to the nerve cord alone but they did get evidence that the leech body wall liberated acetylcholine when the nerve cord was stimulated. The crop and gizzard of the earthworm show spontaneous rhythmic contractions and acetylcholine at 10^{-9} superimposes a slow contraction on this rhythm (332). When the preparations are from earthworms which have been cooled the spontaneous contractions are absent but the response to acetylcholine persists; the ACh effect on the gut is abolished by atropine. A spontaneously active preparation liberates into bathing fluid a substance which may be acetylcholine (4). From the preceding evidence and other contributing details it is likely that acetylcholine has something to do with neuromuscular excitation in somatic and visceral muscles of annelids.

Molluscs appear to differ much in their responses. For example, the slow adductor muscle of Pecten is not stimulated by acetylcholine (26), whereas the retractor muscle of the buccal mass in Helix does respond to the drug (253). In the cephalopods Octopus and Eledone acetylcholine and eserine have no effect on the stellate ganglion, the neuromuscular response of the mantle is not increased by eserine but the mantle muscle is sensitive to acetylcholine and removal of the stellate ganglion greatly increases the mantle sensitivity (12, 15). There was no increase by eserine of responses of foot and siphon muscles of Buccinium and Mya (16). Bacq (12) perfused the mantle of Eledone with eserinized saline; the perfusate contained acetylcholine but stimulation of the mantle nerve did not increase its Ach content.

In the holothurian Stichopus the longitudinal muscles are sensitive to acetylcholine, responding to dilutions of 10^{-7} without eserine and to 10^{-9} after eserine (13). The mantle muscle of the ascidian Cynthia is stimulated by acetylcholine but the response is not potentiated by eserine (15). Bullock (personal communication) found that spontaneous contractions of the proboscis of the enteropneust, Saccoglossus, were greatly increased by acetylcholine. That this sensitivity is not a property of all nerve nets was shown by the fact that the contractions of medusae were not affected by acetylcholine or eserine. Muscles of the actinians Calliactis and Metridium were also insensitive to acetylcholine 10^{-4}, even after eserinization (12). In general, in the above groups of animals there is a rough correlation between response to acetylcholine and the existence of cholinesterase.

Among the arthropods, on the other hand, except for heart and gut there is no clear evidence for any function of acetylcholine. DuBuy (87) stated that the muscles of the crayfish claw are insensitive to acetylcholine; this has been confirmed by Bacq (11) and by Ellis, Thienes and Wiersma (92). The last named authors tested a variety of drugs and ions upon crustacean neuromuscular preparations and could find no evidence for humoral transmission. Katz (166) and Waterman (315) had found an inverse relation between the response of crustacean muscle and magnesium content of the bathing saline but Ellis et al. (92) did not confirm this. There seems to be agreement among all these writers that curare, eserine, and acetylcholine have no effect upon neuromuscular responses of crustaceans.

When applied to arthropod central nervous systems acetylcholine is also ineffective either with or without eserine except at concentrations as high as 10^{-3} in the crayfish (245) and in the cockroach (263). Bacq (11) injected large amounts (up to 40 mgm.) of acetylcholine into a crab (Carcinus and Palinurus) and observed no changes in reflex activity. Welsh and Haskin (318) found a facilitation of autotomy in crabs injected with eserine or acetylcholine.

Synaptic transmission in the last abdominal ganglion of the crayfish was studied by Prosser (243, 245). He stimulated sensory hairs on the uropods and telson mechanically and recorded simultaneously the impulses on both sides of the ganglion. Flexion of one hair sent one impulse into the ganglion. Stimulation of several adjacent hairs, usually 4, was necessary to elicit one efferent impulse, i.e., spatial summation was required. Ganglionic delay for the fastest units was 2–5 msecs. The sensory endings followed repetitive stimulation up to 100 per second while the synapse failed to transmit faster than 10 per second. Paired stimuli showed a non-functional recovery period in the ganglion of 0.1 sec. No after-discharge in the efferent response was ever observed. A very different arrangement exists in the last abdominal ganglion of the cockroach where some sensory fibers pass directly through the ganglion while others synapse with giant neurones (250). The synaptic delay of the latter is 1–2 msec. When the sensory fibers were stimulated repetitively a critical frequency was reached (40–50/sec. at maximal intensities and 15/sec. at submaximal intensities) where the postganglionic response diminished and its latency increased. Recovery of this response could then be brought about by suddenly increasing either the frequency or the intensity, or by interpolating a single extra stimulus on the

afferent side. Temporal summation occurred only when the synapse was in an adapted state of diminished transmission. Apparently a single sensory impulse was effective, hence no spatial summation was needed, but a given giant neurone could be excited by one of several converging sensory fibers.

Prosser tested the effects of various drugs and salts upon ganglionic transmission in the crayfish preparation (245). Acetylcholine, physostigmine (eserine) and prostigmine were without effect upon summation or latency of the efferent response. Treatment of the ganglion with a saline low in potassium did increase facilitation while high potassium decreased it reversibly. The effect of potassium upon the asynchronous "spontaneous" activity of the nerve cord differed in intact and isolated cords (245, 247a, 264). When the cord was in situ, or within a half hour after removal from the animal, low potassium increased "spontaneous" activity while high potassium initially increased and then depressed it. After a half hour of isolation in saline the potassium effect was reversed so that low potassium decreased "spontaneous" activity. Neither stimulating nor depressing action of low potassium was antagonized by low calcium and only the depressing effect of high potassium was antagonized by high calcium. Roeder (265) confirmed these findings and added the important fact that if acetylcholine is added to the saline in which the isolated nerve cord is kept the original response of the intact preparation to potassium persists. The lobster nerve cord loses much of its Ach to a bathing medium (279). Thus it may be that the acetylcholine which exists in the nerve cord (285) is not a synaptic transmitter but that it sensitizes the ganglion cells to any depolarizing agents (such as K), and that acetylcholine may be lost rapidly from an isolated cord.

The preceding evidence indicates that cholinergic systems are widespread, certainly in most groups above coelenterates. The functions of acetylcholine in annelids may be more similar to its functions in vertebrates than in molluscs and arthropods. The meaning of the high cholinesterase and acetylcholine concentrations in arthropod and cephalopod nervous systems is not clear.

Various invertebrates produce other pharmacologically active agents besides acetylcholine, but their effects have not been much studied. In the leech ventral nerve cord there are chromaffine cells which may produce adrenalin (112). Similar cells occur in the Limulus heart (R. Snider, personal communication). Adrenalin stimulates the visceral musculature of some invertebrates (Holothuria (31), earthworm (44)). A possible synergistic action between low concentrations of adrenalin and acetylcholine has not been investigated, nor has the occurrence of adrenalin among lower invertebrates. We mentioned previously the cells of the stellate ganglion of Sepia, which seem to have become endocrine (335). The Scharrers (273) have described neurosecretory cells in the brains of Nemerteans, Annelids, Molluscs, Arthropods and vertebrates.

CONCLUSIONS

The comparative physiology of nervous systems gives less information for tracing phylogenetic relations than does the comparative physiology of many

other systems. This is because (1) the basic nature of nervous conduction is a cellular problem, and (2) most of the general nervous properties evolved very early. In the coelenterate nerve net for example there is already a tendency for increasing speed by through-fiber tracts, there is facilitation in such high degree that it determines a sort of polarity. Inhibition in the sense of an inhibitory state and central antagonism with reciprocal innervation of muscles are present in annelids and, assuming phylogenetic bifurcation below annelids to the two main lines of descent, inhibition may well be found among flatworms.

The use of a thick myelin sheath and nodes is a vertebrate development; syncytial giant fibers probably arose twice—those with contributing cells scattered along the nervous system in the annelid-crustacean line, and those with contributing cells grouped together in the cephalopod molluscs. Unicellular giant fibers are large neurones and grade into ordinary ones; such enlargement of neurones has occurred many times.

It is not clear why peripheral conducting systems or subepidermal networks have been replaced so completely by local reflex centers. It is quite possible that the subepidermal plexus in the earthworm, in molluscan feet (but not in echinoderms) is even now vestigial.

One is impressed with the closely interconnected evolution of central nervous and neuromuscular mechanisms. In the coelenterate nerve net system there is little distinction as to whether facilitation is neuro-neural or neuro-muscular. Some of the so-called peripheral conduction in worms may be muscular. In the arthropods the neuromuscular junction with multiple innervation, facilitation, inhibition becomes more important than the central nervous system in grading motion.

As one passes toward the most successful or "higher" invertebrates one sees increasing headwardness in the nervous system. The sensory basis for this is clear, the integrative basis less so. Learning in the sense of a reversal of a stereotyped response is probably a property of all animals whether they have a nervous system or not, as Jennings has pointed out. Many of the alterations in feeding reactions obtained in coelenterates are undoubtedly sensory adaptation phenomena. In the earthworm conditioning was carried out by the chain of ventral ganglia. Only in the verticalis complex of cephalopods, in the corpora pedunculata of certain insects (and possibly in those of lower arthropods and polychaete worms) is there anything comparable to the association areas of a vertebrate brain. Technical difficulties of small size and fragility have prevented the study that these structures suggest.

Not enough work has been done with pharmacologically active agents in invertebrates to draw conclusions regarding chemical mediators. It is likely that there are cholinergic nerves beginning with flatworms and possibly with coelenterates.

Invertebrate nerves offer many useful preparations for the cellular physiologist. A few examples are: giant fibers for studying membrane phenomena, nerve fibers which remain depolarized for minutes (Crustacea), neuromuscular junctions in which facilitation persists for many seconds (coelenterates, annelids),

inhibitory nerve fibers that can be isolated from excitatory fibers, and muscle fibers which receive from two to five nerve axons each of which elicits a different response.

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THE PHARMACOLOGY OF THE VERATRUM ALKALOIDS

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A review of the pharmacology of the veratrum alkaloids is justified at the present time by several significant advances. The chemical nature of these compounds has been considerably clarified by recent work. The fact that they bear some resemblance to the cardiac glycosides has contributed to the interest in the action of the veratrum alkaloids on the heart. The clinical use of crude preparations containing veratrum alkaloids has undergone a revival in obstetrical clinics, where eclampsia is again treated with these substances. Parallel with this development there has been a revival of interest in the circulatory action of the veratrum alkaloids, and considerable clarification of certain circulatory phenomena has been achieved. Finally, as a result of improved techniques and new data, the interpretation of the action of the veratrum alkaloids upon nerve and muscle has changed radically since this subject was last reviewed.

Because of the emphasis on these advances, this review is of necessity different from previous reviews (Gasser, 83; Querido, 203; Boehm, 27; Biberfeld, 19). Hence some phases of the subject may better be pursued there than here. The medical reader interested in the therapeutic aspect must be referred to the original clinical literature. Although occasional reference is made to important clinical observations, the clinical use of veratrum alkaloids will not be discussed, as it has not yielded fundamental contributions.

One point seems of particular importance for future experimental work. To assure a steady advance in the understanding of the action of the veratrum alkaloids, it is indispensable that pure alkaloids be used instead of alkaloid mixtures whenever this is feasible. This alone will make possible a complete pharmacological analysis. Individual veratrum alkaloids differ markedly in their biological action. Hence the use of pure substances is imperative in physiological work in which veratrum alkaloids are employed as pharmacological tools. Incidentally, only the study of the pure veratrum alkaloids will permit a correct evaluation of the practical usefulness of the individual alkaloids as well as of their mixtures, and thus make possible the development of a rational basis for their clinical use.

I. THE CHEMISTRY OF THE VERATRUM ALKALOIDS. The veratrum alkaloids are obtained from liliaceous plants belonging to the sub-order Melanthaceae. The species most thoroughly investigated are *Veratrum album*, Linn., native to Europe; *Veratrum viride*, Aiton, native to the United States and Canada; and *Schoenocaulon officinale*, Gray, also called *Asagroca officinalis*, Lindley, or *Veratrum Sabadilla*, Retz., the Mexican or West Indian Sabadilla.[1]

[1] The name hellebore is wrongly applied to the Veratrum species or to drugs obtained from these plants, as the Helleborus species belong to the family Ranunculaceae. The incorrect use of the name hellébore, therefore, should be discontinued in the scientific literature.

Alkaloids are probably present in all parts of these plants. The sources from which the chemically known substances have usually been obtained in the past are the roots and rhizomes of *Veratrum album* and *Veratrum viride*, and the seeds of *Schoenocaulon officinale*, the "Sabadilla seeds."

The chemistry of the veratrum alkaloids is now sufficiently well known to make possible the recognition of several well-defined pure substances which are probably built upon a common fundamental structure.

Veratrum album and *Veratrum viride* were found by various authors (42, 128, 132, 133, 197, 198, 199, 200, 225, 226, 227, 234, 236, 262, 264) to yield some or all of the following crystalline alkaloids: protoveratrine, germerine, jervine, rubijervine, pseudojervine, and veratrosine. The seeds of *Schoenocaulon officinale* (24, 30, 76, 119, 172, 263) yield the crystalline cevadine, the amorphous veratridine, and small amounts of the crystalline cevine. Table 1 contains the characteristics of the important alkaloids which are present in the plant, or, like protoverine, protoveratridine, germine, and veratramine, can be obtained as hydrolytic products.

Some of these alkaloids are ester alkaloids which by hydrolysis under suitable conditions can be split into a basic part, or alkamine, and one or more organic acids.

$$\text{Protoveratrine} + 3H_2O \rightarrow \text{Protoverine} + \begin{matrix}\text{acetic acid}\\ \text{methyl-ethyl-acetic acid}\\ \text{methyl-ethyl-glycolic acid}\end{matrix}$$

$$\text{Germerine} + 2H_2O \rightarrow \text{Germine} + \begin{matrix}\text{methyl-ethyl-acetic acid}\\ \text{methyl-ethyl-glycolic acid}\end{matrix}$$

$$\text{Veratridine} + H_2O \rightarrow \text{Cevine} + \text{veratric acid}$$

$$\text{Cevadine} + H_2O \rightarrow \text{Cevine} + \text{tiglic acid (or angelic acid)}$$

The ester alkaloid protoveratridine, according to Poethke (198), is a decomposition product of germerine, the hydrolysis of which may occur in two stages:

$$\text{Germerine} + H_2O \rightarrow \text{Protoveratridine} + \text{methyl-ethyl-glycolic acid}$$

$$\text{Protoveratridine} + H_2O \rightarrow \text{Germine} + \text{methyl-ethyl-acetic acid}$$

Jervine, rubijervine, pseudojervine, veratramine, and veratrosine are non-ester alkaloids, or alkamines, like protoverine, germine, and cevine. Pseudojervine and veratrosine differ from the other alkamines in that they are glycosidic alkaloids (132, 133). On hydrolysis, they split into d-glucose and the respective alkamines, jervine and veratramine, according to the following formulae:

$$\underset{\text{Pseudojervine}}{C_{33}H_{49}O_8N} + H_2O \rightarrow \underset{\text{jervine}}{C_{27}H_{39}O_2N} + \underset{\text{d-glucose}}{C_6H_{12}O_6}$$

$$\underset{\text{Veratrosine}}{C_{33}H_{49}O_7N} + H_2O \rightarrow \underset{\text{veratramine}}{C_{27}H_{39}O_2N} + \underset{\text{d-glucose}}{C_6H_{12}O_6}$$

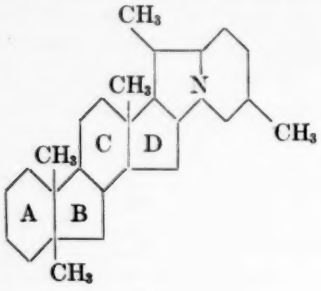
TABLE 1

The empirical formulae, optical rotations and melting points of the known veratrum alkaloids

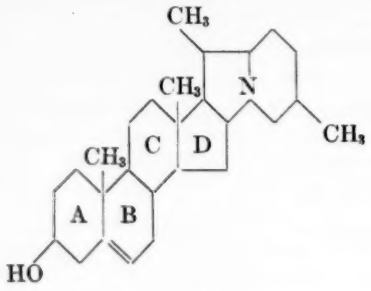
NAME	EMPIRICAL FORMULA	ROTATION	MELTING POINT
Protoveratrine	$C_{39}H_{61}O_{13}N$ (42)	$[\alpha]_D^{28} = -8.5°$ (c = 1.99 in chloroform) (127)	Discolors above 270°; gradually decomposes at 273–276° (uncorr.) (42)
Germerine	$C_{37}H_{59}O_{11}N$ (45)	$[\alpha]_D^{20} = +10.8°$ (c = 1.99* in chloroform) (198)	193–195° (corr.); melts with decomposition (197)
Veratridine	$C_{36}H_{51}O_{11}N$ (24)	$[\alpha]_D^{25} = +8.0°$ (c = 4.0 in 96% ethanol) (24)	Softens when heated and melts over range of 160–180° (24)
Cevadine	$C_{32}H_{49}O_9N$ (172, 263)	$[\alpha]_D^{17} = +12.5°$ (c = 1.19 in ethanol) (172)	199–201° (24)
Protoveratridine	$C_{32}H_{51}O_9N$ (45)		266–267° (corr.). Melts with decomposition (198)
Protoverine	$C_{27}H_{43}O_9N$ (131)	$[\alpha]_D^{25} = -12.0°$ (c = 0.69 in pyridine) (131)	No real melting point. Gradually softens to a resin between 195 and 200° (131)
Germine	$C_{27}H_{43}O_8N$ (45)	$[\alpha]_D^{25} = +5.0°$ (c = 1.03 in 95% ethanol) (45)	Gradually softens to resin between about 163–173°; finally melts on heating to about 220° (45)
Cevine	$C_{27}H_{43}O_8N$ (24, 76, 172)	$[\alpha]_D = -17.52°$ (c = 2.34 in ethanol) (172)	No sharp melting point; sinters at 155–160°, becomes resinous at 165–170°, and is melted at 195–200° (76)
Rubijervine	$C_{27}H_{43}O_2N$ (129)	$[\alpha]_D^{25} = +19.0°$ (c = 1.0 in ethanol) (129)	240–242° (uncorr.) (129)
Jervine	$C_{27}H_{39}O_3N$ (130)	$[\alpha]_D^{23} = -147.0°$ (c = 1.04 in ethanol) (130)	237–238° (uncorr.) (130)
Veratramine	$C_{27}H_{39}O_2N$ (132, 133)	$[\alpha]_D^{27} = -69.0°$ (c = 0.98 in methanol) (133)	Melts at 204–207°C. after preliminary sintering (133)
Pseudojervine	$C_{33}H_{49}O_8N$ (199)	$[\alpha]_D^{20} = -13.0°$ (c = 0.4 in ethanol chloroform (199)	304–305.5° (corr.) (199)
Veratrosine	$C_{33}H_{49}O_7N$ (132, 133)	$[\alpha]_D^{25} = -53.0°$ (c = 0.255 in mixture of chloroform and 95% ethanol, equal volumes) (132)	Discoloration and sintering especially above 230°, softens above 235°, finally becomes a colored effervescent mass at 242–243° (132)

* Germerine + 1 H_2O.

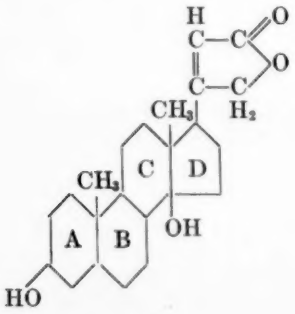
The chemical evidence suggests that the alkamines protoverine, germine, cevine, jervine, rubijervine and veratramine are C_{27} compounds and are built upon a regular or modified sterol structure. The analytical data support the assumption that the veratrum alkamines are closely related to the alkaloidal aglycones of the solanum species as well as to the aglycones of the cardiac glycosides (44, 46). The structural formulae illustrate the relation between the suggested ring system of cevine (I) (25, 42, 129, 223), the structure of solanidine (II) (46, 201, 213), and the structure of digitoxigenin (III) (79, 194).



I



II



III

The possibility persists that ring B of the alkamines other than rubijervine may be 5-membered. The heterocyclic portion of the molecule in the tertiary bases has been established to be an octahydropyrrocoline derivative (251). The nature of the nitrogen containing portion of the molecule of jervine and of veratramine, which are secondary amines (133), remains to be determined. All of the oxygen atoms are probably contained in hydroxyl or keto groups, the position of which is not yet ascertained (46).

That the chemical structure as formulated in (I) is correct and that the relation with solanidine holds has been confirmed by the conversion of sarsasapogenin to allosolanidanol-(3β) by Uhle and Jacobs (251).

For a valid interpretation of the biological work conducted in the past with

veratrum alkaloids it is important to recognize that the name veratrine[2] usually refers to a mixture of alkaloids obtained from Semen Sabadilla (30, 119) and consisting largely of veratridine, cevadine, and a small amount of cevine. The relative proportion of the individual alkaloids apparently varies from batch to batch and the degree of variation is unknown. The extracts of *Veratrum viride* or *Veratrum album* contain a large number of alkaloids, a few of which are chemically known, while the bulk of the alkaloidal material obtained from these plants remains to be separated and identified (see table 2).

As the habitat of the plant has a marked influence upon the composition of the alkaloidal mixture (197, 198, 199, 200, 225, 226, 234) in the various parts of the plant, it cannot be expected that extracts, obtained from specimens of the same species but grown in different locations, should have the same pharmacological properties.

TABLE 2

The alkaloids of Jugoslavian Veratrum album (Poethke, 197)

	ROOTS*		RHIZOMES*		LEAF BASES*	
	Grams	% of total	Grams	% of total	Grams	% of total
Total alkaloids	6.0	100	9.17	100	4.47	100
Protoveratrine	1.05†	17.5	1.33†	14.5	0.54	12.1
Germerine	0.45†	7.5	1.26†	13.7	0.8	17.9
Jervine	0.2	3.3	0.94	10.3	0.03	0.7
Rubijervine	0.2	3.3	0.04	0.4		
Pseudojervine			0.6	6.5		
Amorphous alkaloids	4.1	68.3	5.0	54.5	3.1	69.3

* One kilogram of dry powder was used to obtain the values.

† These are approximate figures adapted from Poethke's data on pure protoveratrine and germerine, and on mixtures of the two alkaloids.

It is obvious, therefore, that neither the name veratrine (usually used for the total alkaloids from *Schoenocaulon officinale*), nor the name veratrum (usually used for extracts containing an unknown number of the total alkaloids from *Veratrum album* or *Veratrum viride*) refer to well-defined preparations of constant composition. But even in many cases in the past where it was implied by the author that pure substances were used for the experimental work, this must be considered doubtful unless specific data can be found indicating the identity of the veratrum alkaloids.

II. TOXICITY. QUANTITATIVE AND QUALITATIVE DIFFERENCES AMONG THE VERATRUM ALKALOIDS. The naturally occurring ester alkaloids of the veratrum group are more powerful pharmacological agents than are the alkamines. This calls to mind similar pharmacological facts, for example; the difference between

[2] This is the only justifiable use of the name; Veratrinum crystallisatum purissimum Merck is assumed to be cevadine.

the solanaceous ester alkaloids, atropine and scopolamine, and their respective alkamines, tropine and scopine, in regard to their influence upon impulse transmission to autonomic effectors; or the difference in the pharmacological action of choline esters and choline. While Falk (64) had already recognized that a considerable difference in toxicity exists between cevadine and cevine, quantitative toxicity experiments with pure alkaloids were made only recently (103, 154, 182) (see table 3). Protoveratrine so far has been shown to be the most toxic of the veratrum alkaloids. The LD 50 in mice upon intravenous administration shows it to be on a molar basis 10 times more toxic than veratridine and 6000 times more potent than its own alkamine protoverine.

TABLE 3

The toxicity of the veratrum alkaloids (LD 50) in vertebrate animals

ANIMAL	WAY OF ADMINISTRATION	SUBSTANCE	MOL. WEIGHT	LD 50		REFERENCE
				mgm./kgm.	Micromoles per kgm.	
Rana esculanta	Subcutaneous	Protoveratrine	751	4.5	6.0	(103)
Rana esculanta	Subcutaneous	Germerine	679	9.0	13.2	(103)
Rana temporaria	Subcutaneous	Protoveratrine	751	13.5	17.9	(103)
Rana temporaria	Subcutaneous	Germerine	679	20.0	29.4	(103)
Rat	Oral	Protoveratrine	751	5.0	6.7	(103)
Rat	Oral	Germerine	679	30.0	44.1	(103)
Rat	Subcutaneous	Protoveratrine	751	0.6	0.8	(103)
Rat	Subcutaneous	Germerine	679	3.7	5.4	(103)
Rat	Intraperitoneal	Veratridine	673	3.5	5.2	(182)
Rat	Intraperitoneal	Cevine	509	67.0	131.0	(182)
Mouse	Intravenous	Protoveratrine	751	0.048	0.06	(154)
Mouse	Intravenous	Veratridine	673	0.42	0.63	(154)
Mouse	Intravenous	Cevadine*	591	1.0	1.7	(153)
Mouse	Intravenous	Jervine	425	9.3	21.9	(154)
Mouse	Intravenous	Rubijervine	413	70.0	170.0	(154)
Mouse	Intravenous	Cevine	509	87.0	170.0	(154)
Mouse	Intravenous	Germine	509	139.0	274.0	(154)
Mouse	Intravenous	Protoverine	525	194.0	367.0	(154)

* This was the alkaloid of Ikawa et al. (113) and may not have been pure.

The alkamines differ considerably in toxicity among themselves, the most toxic being jervine. Chemically, jervine is a secondary amine while the others (with the exception of veratramine, which has not been studied) are tertiary amines. Also, jervine is the strongest levorotatory compound of the alkamine group so far investigated. Cevine is distinctly more effective than its isomer germine.

Apart from and independent of the genuine toxicity of the alkamines, the toxicity of the ester alkaloids obviously is dependent upon the number of acyl groups in the molecule as well as upon the nature and probably also the position of the individual acyl groups. This can be concluded from the limited and inaccurate toxicity studies on vertebrate animals reported in the literature (table 4).

It follows from the data of Heinz (109) (see table 4) that the introduction of an acetyl group and still more of a benzoyl group into the molecule of cevadine decreases notably toxicity in frogs as well as in rabbits. Dibenzoyl cevine is still weaker in effect than benzoyl cevadine. Haas' observations (103) on rats (tables 3 and 4) with subcutaneous injections of germerine, protoveratridine and germine indicate clearly that the removal of the methyl-ethyl-glycolic acid from germerine reduces toxicity to $\frac{1}{250}$; and protoveratridine, the methyl-ethyl-acetic acid ester

TABLE 4

*The toxicity of the veratrum alkaloids (approximate lethal doses) in vertebrate animals**

ANIMAL	SUBSTANCE	LETHAL DOSE†		REFERENCE
		mgm./animal	mgm./kgm.‡	
Rana	Protoveratrine	0.2	6.0	(256)
Rana	Cevadine	0.05	1.5	(109)
Rana	Cevadine (Sulfate?)		**7.0**	(195)
Rana escul. and temp.	Cevadine	0.5–1.0	15.0–30.0	(166)
Rana	Acetyl Cevadine Hydrochloride	1.0	30.0	(109)
Rana	Benzoyl Cevadine	>10.0	>300.0	(109)
Rana	Dibenzoyl Cevine Acetate	20.0	600.0	(109)
Rana escul.	Protoveratridine		**>750.0**	(103)
Rana escul.	Germine		**250.0–500.0**	(103)
Guinea pig	Cevadine	0.5		(109)
Rabbit	Protoveratrine		**0.11**	(256)
Rabbit	Cevadine	1.0	0.5	(109)
Rabbit	Cevadine	2.7–4.2	1.3	(166)
Rabbit	Acetyl Cevadine Hydrochloride	50.0	25.0	(109)
Rabbit	Dibenzoyl Cevine Acetate	>100.0§	>50.0	(109)
Rat	Protoveratridine		**>1000.0**	(103)
Rat	Germine		**2000.0**	(103)

* In all experiments the way of administration was by subcutaneous injection.

† Unless otherwise mentioned the dosages refer to the base and not to the salt.

‡ The boldface figures for lethal dose in mgm./kgm. were those actually reported. The others were calculated from the reported values for the whole animal to get an approximate dose per kgm.: for the frog by multiplying by 30, assuming an average weight of 33 grams per frog; for the rabbit by dividing by 2, assuming an average weight of 2 kgm. per rabbit.

§ This dose caused only slight depression of the central nervous system.

of germine, is but little more toxic than the alkamine germine itself. Experiments on *Musca Domestica* L. (119) show veratridine to be a more potent insecticide than cevadine; while cevine dibenzoate and cevine have no insecticidal action.

The marked qualitative differences between the effects of the individual veratrum alkaloids are well illustrated by the observations on mice that died after the administration under the same conditions of an intravenous dose close to the LD 50 (154) (see table 3). The results with two ester alkaloids and five alkamines were as follows:

Protoveratrine: (relative LD 50 = 1) For a period of 90 seconds after the injection no signs of poisoning could be recognized. A period of flaccid paralysis then followed succeeded in some cases by irregular respiration, in others by convulsions. Death occurred within 2 to 4 minutes.

Veratridine: (LD 50 relative to protoveratrine = 10) Immediately after the injection respiration was decreased in rate and markedly disturbed in rhythm. Death occurred with signs of respiratory failure within 1 to 4 minutes. In some cases convulsions preceded death.

Jervine: (relative LD 50 = 350) After a latent period of 30 seconds, no appreciable sign of poisoning was noticed; marked motor excitation then started, leading to repeated convulsive seizures. Death occurred in a convulsive attack within 10 minutes.

Rubijervine: (relative LD 50 = 3000) Immediately after the injection there was either mild motor excitation or a decrease in motor activity. In some animals there was respiratory depression, in others, the outstanding symptoms were fits of tremor or convulsive seizures. Death occurred within 30 seconds to 2 minutes in a convulsive attack or under the signs of respiratory failure.

Cevine: (relative LD 50 = 3000) Tremor and convulsive movements started immediately after the injection. Within a few minutes the tremor became continuous, the animals lay prone, with legs and tail extended. There was a very marked increase in motor excitability to touch and especially to sharp, high-frequency noises. Animals showing these signs died as a rule within 30 to 180 minutes. A few animals had moderately severe convulsive seizures and a slow and labored respiration starting immediately after the injection. These animals died within two minutes.

Germine: (relative LD 50 = 4500) Within a few seconds after the injection the animals began jumping about and had convulsive seizures. Within 1 to 3 minutes they became unable to stand, lay on their sides and had continuous, severe convulsions. Breathing was slow and irregular. Death occurred within 1 to 3 minutes.

Protoverine: (relative LD 50 = 6000) After a latent period of about 30 seconds there were brief fits of coarse tremor, especially of the head. In most animals, death occurred about 5 minutes after the injection following a brief period of convulsive movements. Some animals had severe jerking movements, their respiration became slow and gasping; they died within 1 to 3 minutes.

Other quantitative and qualitative differences among the alkaloids will be referred to in connection with the discussion of their effect upon the various physiological systems.

III. The absorption, distribution and elimination of the veratrum alkaloids. No adequate quantitative data are available on the absorption of the veratrum alkaloids from the mucosa of the intestinal tract or from other sites of application to the mammalian organism. The experiments of Haas (103) on rats (see table 3) with oral and subcutaneous administration of protoveratrine and germerine indicate that approximately 90 per cent of the oral LD 50 becomes inactivated in some way during the passage from the intestinal tract to the sites of action responsible for the lethal effect, as the subcutaneous LD 50 of both alkaloids is only $\frac{1}{8}$ of the oral dose.

The distribution of the alkaloids in the intact animal organism has not been studied. In the isolated heart of a marine snail, *Aplysia limacina*, L. which can take up relatively huge amounts of veratrine without being killed, Straub (243, 244) investigated the distribution between blood and heart tissue. He exposed the heart to varying concentrations of veratrine in the blood and determined the final concentration in blood and heart by a comparison of the relative toxicity to

frogs of blood and of extracts of the heart tissue. Straub concluded that a considerable accumulation of veratrine occurs readily in the heart muscle tissue. Equilibria are reached with low concentrations in the blood and high concentrations in the heart tissue. With increasing concentrations in the blood, the ratio of tissue concentration to blood concentration decreases. Veratrine can be washed out of the muscle tissue of the Aplysia heart. According to Freundlich (77), Straub's data indicate that veratrine is adsorbed to the cell surfaces.

Straub could not recognize any destruction of veratrine, although the alkaloid mixture was kept for 2 to 4 days in the tissue of the Aplysia heart.

The fate of the veratrum alkaloids in the mammalian organism is unknown except for the scant evidence that a small fraction appears to be excreted by the kidney (202, 249), and by some of the excretory organs of the gastro-intestinal tract (118).

IV. The action of the vertatrum alkaloids upon the respiratory system. In the normal as well as in the anesthetized mammal all pure ester alkaloids of the veratrum series so far studied cause a decrease in the rate of respiration. On the other hand, some of the veratrum alkamines even in large doses appear to be devoid of this property (154, 182).

The smallest active doses of the ester alkaloids, cevadine (109, 166), veratridine (182, 190), germerine (103) and protoveratrine (154, 256) bring about a transient slowing of respiration; larger doses lead to respiratory stoppage increasing in length proportional to the dose. Qualitatively and quantitatively the respiratory effects of cevadine, germerine and veratridine appear to be similar. In a large measure the respiratory arrest contributes to the lethal effect of fatal amounts.

The minimal doses effective upon respiration are somewhat higher than the minimal doses influencing the circulatory system (section V, A and B). In the anesthetized dog (sodium pentobarbital) (190) no respiratory effect or occasionally a slight increase in respiratory rate is observed with the minimal doses of veratridine leading to a significant cardiodeceleration, or vasodilatation. With protoveratrine this difference is very pronounced. Doses which in the dog under pentobarbital anesthesia cause marked cardiodeceleration and vasodilatation are without apparent respiratory action (154). The mechanism involved in the respiratory response to small doses appears to differ from that brought into action by large doses. This is indicated by the experiments showing the rôle of the vagus nerves.

Atropine sulfate in doses of 1 mgm. per kilo in anesthetized dogs does not modify significantly the respiratory action of cardiodecelerator and depressor doses of veratridine. Vagotomy almost completely abolishes or prevents the respiratory action (190). After vagotomy larger doses, especially those designated in section V, D as pressor and cardioaccelerator doses, still cause respiratory arrest (190).

The experiments of von Bezold and Hirt (18) with veratrine and of Cramer (47) with veratrum viride make it obvious that part of the respiratory response is reflex in nature, while part appears to be due to central action. The reflex action

can be demonstrated with small doses and in its full strength is difficult to obtain repeatedly, as the receptor mechanism appears to become readily irresponsive. After this, doses larger than the minimal effective doses can be injected without effect, while further increase in dosage again leads to respiratory depression which seems to be independent of the functioning of the vagus nerves.

Receptor areas for the inhibitory respiratory reflex are in areas served by the vagus nerves and probably are located in the viscera of the chest. Exclusion of the depressor nerves in the rabbit does not abolish the response, while cutting the vagus nerves prevents the reflex respiratory action even when the depressor nerves are left intact (18). Similarly, cold block of the vagus in the cat (139) prevents the reflex respiratory effect as well as the vasodepressor effect of veratrine, while both promptly appear when conduction of the vagus nerves is restored by warming.

No comprehensive and comparative study of the respiratory action of the pure veratrum alkaloids has been carried out so far. The sites of action are not definitely established. While it is unlikely that the doses leading to the reflex stoppage of respiration have a direct effect upon the striated muscles of the respiratory apparatus, this possibility exists with doses leading to death under the symptoms of respiratory failure. Evidence is lacking, however, as to whether the intercostal muscles and the diaphragm, or their nerves are directly involved in the fatal respiratory disturbance.

V. THE ACTION OF THE VERATRUM ALKALOIDS UPON THE CIRCULATORY SYSTEM. The studies on the circulatory action of the veratrum alkaloids in mammals have been concerned mainly with changes in blood pressure and heart rate in the intact animal and with effects upon the isolated heart. On the basis of the available evidence a complete analysis of the very complex circulatory changes is not yet possible.

It will facilitate the understanding of the mechanisms involved in the action upon blood pressure and heart rate if three at present clearly distinguishable effects are discussed in detail. They are: first, the depressor effect; second, the cardiodecelerator effect; and third, the pressor and cardioaccelerator effect. The first and second as described in the following chapters can clearly be recognized if small doses of the veratrum alkaloids are used; they are not demonstrable unless the vagal mechanism is intact. The third, on the other hand, can best be observed if the veratrum alkaloids are administered in large doses and if the vagus is partially blocked by giving atropine, or if the vagus nerves are completely interrupted, as for instance by cold block or by severing the nerves in the neck.

A. *The Depressor Effect of Small Doses of the Veratrum Alkaloids.* 1. *Blood pressure decrease and vasodilatation.* A transient decrease of blood pressure can be observed in mammals (man, dog, cat, rabbit) not only with veratrine, the alkaloid mixture from *Schoenocaulon officinale* (18, 138, 139), and with the alkaloid mixtures from *Veratrum viride* (47, 174, 196, 259, 261), *Veratrum album* (38, 39), and other *Veratrum* species (104), but also with the pure ester alkaloids, cevadine (166, 196), veratridine (182, 190), protoveratrine (154, 256), and germerine (103).

The active dosage range in dogs of 15 to 20 kgm. body-weight anesthetised with sodium pentobarbital is 0.02 to 0.1 mgm. veratridine (190) or 0.1 to 0.05 mgm. protoveratrine (154) injected intravenously close to the right atrium. Within this range the fall of blood pressure is proportional to the dose. An effect comparable to that of the highest dose of veratridine or protoveratrine can be obtained with the alkamine jervine on the basis of Wood's experiments in dogs (260) if doses of the order of 200 mgm. are injected intravenously. None of the other alkamines has been tried in such large doses. Cevine lacks the characteristic depressor property even in amounts up to 2000 times the minimal effective dose of its ester alkaloid, veratridine (182, 190).

The blood pressure decreasing action characteristic of the veratrum alkaloids is illustrated by the studies in dogs with the dosage range of veratridine (190) and protoveratrine (154) mentioned above. Provided nothing interferes with the normal influence of the vagus nerves upon the circulatory system, there is, after the intravenous injection of veratridine close to the right atrium, a latent period of 4 to 12 seconds (average 7.3 sec. in 8 expts.); mean carotid pressure then falls abruptly, reaches a minimum within 30 seconds, and within 2 to 3 minutes returns to the level of the control period or slightly less. With protoveratrine the blood pressure fall is less abrupt, the minimum is reached within 1 to 3 minutes and the return to a level usually below the normal requires 10 to 30 minutes.

This decrease in blood pressure, as a rule, is accompanied by a decrease in heart rate and, especially after veratridine, by a decrease in rate and amplitude of respiration (section IV). The cardiodecelerator as well as the respiratory response, if intense enough, contribute to the action upon the mean blood pressure. However, artificial respiration (18, 154, 190, 196) which prevents the consequences of the respiratory effect, does not modify significantly the blood pressure response to veratrum alkaloids. The experiments referred to below on the influence of atropine, or of severing of the vagi, upon the depressor effect, show that under conditions where there is little or no effect upon heart rate or respiration, depressor action can still be induced by the veratrum alkaloids. Positive evidence of the independence of the depressor action from cardiodecelerator and from respiratory action is derived from the experiments of Krayer, Wood and Montes (155) on the innervated heart-lung preparation of the dog. In some of these experiments the head was perfused at a constant rate by means of a pump. Only nervous connections existed between the head circulation and the artificially ventilated heart-lung preparation. Administration of veratridine into the heart-lung-circuit led to a decrease in blood pressure in the head circulation (see ref. 155, fig. 4A).

These experiments demonstrate that a diminution of arterial resistance is involved in the blood pressure decrease caused by veratridine. Similarly plethysmographic studies show an increase in the volume of various parts of the mammalian organism coincident with the fall of blood pressure caused by the intravenous injection of veratrine (139) or of veratrum viride (47, 259). Such increases in the capacity of the vascular bed have been observed in the liver (139), intestine (47) and kidney (47) of the cat, and in the hind leg and—although less con-

sistently—in the spleen of the dog (259). This indirect evidence for vasodilatation has recently been substantiated by more direct observations. The retinal arterioles of patients receiving veratrum viride dilate during the blood pressure fall (259). Direct quantitative measurements of blood flow in the femoral arteries of anesthetized dogs by means of a differential manometer show a marked absolute increase in blood flow during the blood pressure decrease caused by the intravenous injection of veratridine (190) or protoveratrine (154).

While vasodilatation has been demonstrated in various areas of the circulatory system of the mammal no direct quantitative measurements of alterations in blood flow have so far been made simultaneously in several areas. It is not known what quantitative changes in the distribution of blood, in venous return, and in cardiac output result from graded doses of any one of the pure veratrum alkaloids.

2. *The nature of the vasodilator response.* During the blood pressure decrease the motor elements of the vessel wall, qualitatively at least, react like those of normal vessels to vasoconstrictor drugs (47) and to physiological constrictor stimuli (47, 139, 174). Thus injection of pituitary extract or epinephrine leads to a prompt rise in blood pressure, and asphyxiation leads to a reduction in the increased liver volume coincident with the blood pressure increase (139).

To decide the question whether the veratrum alkaloids have a direct effect upon the vessel wall or exert all their influence via the nervous system, Krayer and his collaborators (154, 190) carried out perfusion experiments in dogs with veratrine, veratridine, and protoveratrine. One hind leg of a "recipient dog" was perfused from a "donor dog" by connecting one carotid artery of the "donor" with the femoral artery of the "recipient" and returning the blood from the femoral vein of the "recipient dog" to an external jugular vein of the "donor dog." All the remaining vascular connections between the perfused leg and the trunk of the "recipient dog" (with the exception of bone and nerve vessels) were interrupted by means of an ecraseur leaving intact the nerve connections via the main trunks of the mixed nerves. Continuous blood flow measurements were recorded simultaneously from both femoral beds of the "recipient dog." The intravenous injection of the alkaloids into the "recipient dog" outside the perfused leg resulted in a characteristic increase in blood flow in the leg perfused from the "donor dog" as well as in the leg which was in normal vascular and nervous connection with the trunk of the "recipient dog." On the other hand, no response could be obtained by injections of the alkaloids into the arterial blood supply from the "donor dog" to the perfused leg of the "recipient dog." The dosage range of veratridine was 0.01 mgm. to 0.5 mgm. leading to concentrations of from 1:150,000 to 1:3000 during the intra-arterial injection. With protoveratrine the doses ranged between 0.015 mgm. and 0.075 mgm. leading with the lowest dose to approximately 1:100,000, with the highest dose to 1:25,000 in the artery. Thus doses leading to concentrations which might conceivably arrive at the entrance of the femoral vascular bed following the intravenous injection as well as doses many times higher proved ineffective upon direct intra-arterial administration.

Perfusion experiments on isolated vascular beds, undertaken to study the direct

action of the veratrum alkaloids upon the vessels, have not yielded consistent results regarding the vasodilator effect. In the Trendelenburg preparation of the frog a slight increase in perfusion rate was observed (151) with concentrations below 1:100,000; higher concentrations reduced the flow. In the hind legs of mice and rats or in the rabbit ear perfused with Ringer solution the minimal active concentrations of 1:200,000 or above caused only constriction of the vessels. Willson and Smith (259), however, observed increase in perfusion rate of the isolated kidney, ear, or hind leg of the rabbit perfused with Ringer solution when veratrone—a mixture of alkaloids of *Veratrum viride*—was injected. The effect was more consistent when the rate of flow was reduced by the previous administration of epinephrine. These experiments on isolated vascular beds need corroboration with pure veratrum alkaloids.

The perfusion experiments in the innervated femoral bed of dogs justify the conclusion that the vasodilator action of small doses of veratrum alkaloids cannot be explained by a direct influence upon the vessel wall. The action is transmitted to the motor effectors of the vessels predominantly, if not altogether, by way of nervous pathways reaching the femoral vascular bed through the mixed nerves of the leg. The nature of the nerve fibers involved in the reaction has not been identified.

3. *The rôle of the central nervous system.* The rôle of the central nervous system in the vasodepressor response to small doses of veratrum alkaloids is not entirely clarified. There is no conclusive evidence of a direct inhibitory action upon the vasomotor center. Doses of veratrine hydrochloride below 2 micrograms placed into the fourth ventricle of the cat are without any effect, while 2 to 5 micrograms cause increase in blood pressure and acceleration of heart rate. The injection into the vertebral artery of the cat of doses of 10 to 20 micrograms of veratrine hydrochloride is either without effect or is followed by a slight rise in blood pressure and increase in heart rate. When injected into the jugular vein these doses have a distinct vasodepressor and cardiodecelerator action (209). These experiments support the assumption, first put forward by von Bezold and Hirt, that the vasodepressor response to small doses of veratrum alkaloids is not central but reflex. This is borne out by the studies on the afferent pathways and receptor areas involved in the vasodepressor response.

4. *The rôle of the vagus.* The blocking of cholinergic muscarinic effects by atropine in the cat (47, 139) and in the dog (174, 190) does not prevent the fall in blood pressure caused by veratrum viride (47, 174), veratrine (139) or veratridine (190). In the experiments of Moe, Bassett and Krayer (190) in dogs 1 mgm. per kgm. of atropine sulfate largely abolished bradycardia and had no effect upon the respiratory response; increase in femoral arterial flow still occurred and blood pressure fell although not quite as low as in control observations.

Interruption of the conductivity of the vagus nerve by cutting or cooling has a more pronounced effect than atropinization. Veratrum viride was found to be inactive in the cat after cutting the vagus nerves (47). Cold block of the vagus nerves prevented the blood pressure fall after veratrine in the cat (except after the first injections in some experiments) while the drop in pressure promptly occurred

when conduction was restored by warming the nerves (139). Conversely, after veratrine had established blood pressure decrease and increase in liver volume in the cat, cold block of the vagus immediately raised the blood pressure and caused a reduction of the liver volume. Vagotomy below the heart did not prevent the vasodepressor response in the cat (139).

While interruption of the conductivity of the vagus in the neck markedly interferes with the vasodepressor effect, it does not abolish it. In MacNider's experiments (174) with veratrum viride in the dog, the blood pressure did not fall to the same extent after vagotomy as before. In seven out of ten experiments in dogs, Moe, Bassett and Krayer (190) found the depressor response to veratridine markedly diminished by vagotomy but not abolished altogether in any case. This occurred in spite of the fact that in the majority of the experiments bradycardia and respiratory effects were either completely or almost completely prevented. In three experiments of this series the relative vasodilator effect in the femoral arteries was as marked as before vagotomy. Areas not served by the vagus must therefore contribute to the vasodepressor action.

5. *Non vagal afferent pathways.* If veratrine was administered into the circuit of the carotid sinus area of the dog, exteriorized according to Heymans (114), decrease of blood pressure and bradycardia occurred in the dog. Occlusion with lycopodium of the vessels of the glomus caroticum prevented both effects (139). In the rabbit von Bezold and Hirt (18) could not abolish the depressor response to veratrine by cutting the depressor nerves, whereas the response was absent when the vagus nerves were cut and the depressor nerves left unimpaired. While the carotid sinus nerves carry afferent fibers for the vasodepressor reflex, the depressor nerves presumably do not contribute to the reaction.

6. *The receptor areas located in the heart and lungs.* In order to demonstrate that a veratrum alkaloid confined to the heart and lungs can by a nervous mechanism cause the characteristic action in a distant vascular bed, Krayer, Wood and Montes (155) used the innervated heart-lung preparation of the dog. The head was perfused with a pump at a constant rate. No vascular but only nervous connections existed between the head-circuit and the heart-lung-circuit. Placing veratridine into the heart-lung-circuit caused a decrease in blood pressure in the head-circuit.

Jarisch and Richter (140) attempted to localize more precisely the receptor areas in the heart and lungs from which vasodepressor effects can be elicited. They systematically interrupted various sections of the nerve supply of the heart and lungs of the cat and tested whether blood pressure decrease with veratrine could still be obtained. They either cut the nerve branches or, in the immediate vicinity of the epicardium, destroyed the nerve tissue, or interrupted its conductivity by the application of local anesthetics or nerve poisons (procaine, phenol or veratrine) to special areas of the surface of the heart. Afferent pathways for the depressor response were not found in the sympathetic nerves, or in the aortic nerves, but exclusively in the vagi. While there were contributory afferent fibers coming from the lungs (208), the veratrine depressor response originated predominantly in the heart. The pathways mainly run in those branches of the cardiac nerves not carrying efferent inhibitory fibers. Afferent fibers coming

from the region of the ventricles were found to be of great importance for the circulatory reflex. These observations appear to resolve the disagreement between von Bezold and Hirt (18) who assumed that the impulses came from the heart only, and Cramer (47) who suggested that the lungs were their exclusive origin.

7. *Tachyphylaxis.* Veratridine in the intact dog may be injected repeatedly with little or no diminution of depressor, or vasodilator responses, provided the doses are near the minimal effective dose; if larger doses are used, the effect of subsequent doses becomes less or may even be absent. Protoveratrine, unlike veratridine, leads to marked weakening of the response even with the smallest effective doses (154, 190). According to Jarisch and Richter (140), the depressor reflex originating in the heart of the cat is less subject to tachyphylaxis than circulatory reflexes from other receptor areas.

8. *Summary.* The evidence presented points to the following conclusions: The mechanism mainly operative in the vasodepressor response of the mammalian organism to small doses of veratrum alkaloids is a vasodilatation of reflex nature. Most of the afferent pathways of the reflex run in the vagus nerves, none apparently in the aortic nerves and in the sympathetic outflow of the autonomic nervous system. As, in the dog at least, vagal section in most cases reduces the magnitude of the response without abolishing it, areas not served by the vagus must contribute to the effect; the glomus caroticum appears to be one of these. According to Jarisch the most important receptor area for the reflex is in the heart, as von Bezold and Hirt suggested. The evidence for the ventricular origin of an important section of afferent fibers needs corroboration. While there are receptor areas also in the lungs, they are not the exclusive origin of the reflex, as Cramer assumed.

B. *The Cardiodecelerator Effect of Small Doses of the Veratrum Alkaloids.* Small doses of the veratrum alkaloids, which upon intravenous injection in the mammal lead to the reflex vasodepressor action described in the previous chapter, as a rule cause a decrease in heart rate. Within the dosage range stated above for veratridine and protoveratrine in the dog the intensity of the bradycardia is proportional to the dose. There are considerable differences between the two alkaloids regarding the time-characteristics of their cardiodecelerator action. When injection is made into or close to the entrance of the right atrium, veratridine bradycardia (190) begins after a latent period of a few seconds, reaches its maximal intensity within 30 seconds, and lasts up to five minutes. After protoveratrine the latent period lasts between 40 and 120 seconds, bradycardia develops gradually, the minimal heart rate is reached within one to three minutes, and the duration of action extends over ten to thirty minutes or more (154).

1. *Central versus reflex nature of the cardiodecelerator effect.* It has long been known that vagotomy abolishes or prevents the cardiodecelerator effect and that the administration of large doses of atropine greatly reduces the effect. While some of the investigators explain the lowering of the heart rate as due to central vagal stimulation (18, 115, 196), others consider it to be mainly the result of a reflex vagal stimulation (47, 139, 209).

The apparent discrepancy in regard to the central versus reflex nature of the

cardiodecelerator effect has been largely resolved by the experiments of Krayer, Wood and Montes (155) with veratrine and veratridine, and of Krayer, Moe and Mendez (154) with protoveratrine. The innervated heart-lung preparation of the dog was arranged in these experiments in such a way that the head circulation was separated completely from the heart circulation and there were only nervous connections between the two circuits. Doses of five micrograms of veratridine or protoveratrine injected into the heart-lung-circuit (total blood volume approximately 800 cc.) caused a decrease in heart rate of about 50 per cent, which did not occur when the vagi were cut. (In the ordinary heart-lung preparation doses of this order are without effect upon heart rate.) Upon injection into the cephalic circulation, doses of 10 to 70 micrograms of veratridine or 10 to 30 micrograms of protoveratrine also resulted in cardiac slowing, which, as was shown in experiments with veratridine, could still be obtained after denervation of the carotid sinus, although the effect appeared to be less in intensity. The alkamine cevine given in doses of up to approximately 2 mgm. into the heart-lung-circuit, or into the head-circuit, did not affect the heart rate.

Jarisch and Richter (139) report that marked bradycardia could be elicited in the dog, at least with the first doses, when veratrine was placed into the isolated carotid sinus perfused according to Heymans (114).

Provided there are no other receptor areas in the head region of the innervated heart-lung preparation after denervation of the carotid sinus, it must be assumed on the basis of these experiments that the carodiodecelerator action of the veratrum alkaloids in the mammal is caused in part by reflex and in part by central vagal stimulation.

It has been suggested (139) that a reflex inhibition of accelerator impulses may contribute to the cardiodecelerator action in the intact animal. This would explain the much greater difficulty of preventing the bradycardia of small doses of the veratrum alkaloids by atropine as compared to complete interruption of afferent as well as efferent vagal fibers, but definite proof for this assumption is lacking.

2. *Qualitative difference between veratridine and protoveratrine.* In the innervated heart-lung preparation, as in the intact animal, a difference is apparent in the time-characteristics of the cardiodecelerator response when the effect of veratridine is compared with that of protoveratrine. This is noticeable when the alkaloids are administered into the heart-lung-circuit as well as when they are injected into the head circulation. The slowness of the development of maximal bradycardia is particularly striking when protoveratrine is injected into the cephalic circuit.

3. *Tachyphylaxis.* The reflex decrease in heart rate originating in receptor areas of the heart and lungs and of the carotid sinus cannot be obtained repeatedly with the veratrum alkaloids unless doses are used close to the minimal effective dose (47, 139, 154, 155, 190). Larger initial doses diminish or may altogether prevent the response to a subsequent dose. In case of veratridine it has been shown that this must be due mainly to a change in the receptor mechanism, as cephalic administration in the innervated heart-lung preparation still leads to

cardiodeceleration, when the injection into the heart-lung-circuit is no longer effective. The central action on the other hand does not appear to be weakened as readily.

C. *The Bezold Effect.* As has been shown in section V, A and B, the reflex vasodilatation and cardiodeceleration caused by the veratrum alkaloids originates predominantly in the viscera of the chest. According to Jarisch and Richter the most important part of the impulses for the vasodepressor reflex come from the myocardium of the ventricles and are carried exclusively by afferent fibers of the vagus nerves. For this reflex of cardiac origin they (138) propose the name Bezold effect, because it was A. von Bezold who explained by such a reflex mechanism the vasodepressor action of veratrine, which he discovered in 1867.

Von Bezold and Hirt considered the possibility that the effect of the veratrum alkaloids might be due to a change in sensitivity of pressoreceptor endings to their adequate stimuli. The receptors might be specific chemoreceptors, however, and biochemical changes might lead to an increased discharge of the receptors. It is of interest in this connection that the veratrum alkaloids are not the only substances capable of eliciting the Bezold effect. According to Jarisch and his collaborators (137, 142), other substances, especially active principles of *Viscum album*, L., also reveal apparently the same circulatory reflex.

As far as the veratrum alkaloids are concerned, it has been pointed out above that the ester nature appears to be essential for the reaction, and that the mechanism by which the esteralkaloids act is subject to tachyphylaxis. Following the concept of Bacq (11) (section VI, L) Jarisch (135) put forward the hypothesis that the receptors in the heart responsible for the Bezold effect are sensitized by the veratrum alkaloids to the influence of potassium ions released during the activity of the heart muscle. Amann and Jarisch (8) present evidence that in cats the intravenous injection of otherwise ineffective doses of potassium ion causes circulatory effects similar to the Bezold effect, after the animals have been given several doses of 0.05 to 0.1 mgm. of veratrine hydrochloride. The vasodepressor and vasodilator effect can be abolished or prevented by cold block of the vagus and can no longer be elicited after local anesthesia of the epicardium by procaine. Similar effects are obtained with barium ion and rubidium ion, as well as with substances like sodium citrate and sodium oxalate, which shift the potassium-calcium ratio in favor of the potassium ion. Conversely injection of calcium salts has an antagonistic effect.

Extensive corroborative evidence is required to establish the concept of the Bezold effect; especially as Jarisch (135, 138) is inclined to ascribe to it a considerable importance in the control of the distribution of the blood in the mammalian circulatory system under physiological and pathological conditions. It must be emphasized that it is a complicated task to separate experimentally chemo- and pressoreceptor areas in the myocardium of the ventricles from those already recognized in the large vessels or attached to them; and this task cannot yet be considered to have been satisfactorily accomplished.

D. *The Pressor and Cardioaccelerator Effect of the Veratrum Alkaloids.* The

intravenous injection of more than 0.1 mgm. of veratridine in dogs of 15 to 20 kgm. causes a blood pressure fall to very low levels and the pressure may fail to recover (190). After doses of protoveratrine of the same order there is usually a secondary increase in blood pressure above the normal level (154, 256).

1. *The simulated vagus block caused by protoveratrine.* As Watts Eden (256) already observed, protoveratrine leads to a temporary decrease or abolition of the normal response to electrical stimulation of the peripheral end of the severed vagus nerve. Doses of 0.1 to 0.3 mgm. of protoveratrine (144) injected into dogs of 15 to 20 kgm. cause a complete abolition of the response, and recovery is still incomplete after three hours. The electrocardiographic analysis reveals that during vagal stimulation the normal pacemaker is depressed and an ectopic rhythm below the A.V. node is established. Rothberger and Winterberg (222) observed this phenomenon with several substances. In contrast to the action of atropine this effect of protoveratrine is exerted on the automatic properties of the myocardium rather than on either the vagus nerves or the mechanism of humoral transmission. Veratridine in doses of 0.1 to 0.2 mgm. lacks this action. The action of protoveratrine in causing a simulated vagal block may explain in part the weakening at least of the cardiodecelerator response when repeated doses are given.

2. *The time-characteristics of the pressor and cardioaccelerator effect.* After vagotomy doses of veratridine between 0.1 and 0.5 mgm. in the dog still cause a blood pressure decrease, but this becomes less as the dose increases. Doses between 0.5 and 1.0 mgm. after vagotomy, as well as after blocking of the vagus with atropine, cause an increase in carotid pressure up to 50 mm. Hg or more. The pressure begins to rise within 15 to 30 seconds after the intravenous injection close to the right atrium, reaches the maximum within one to three and one half minutes, and gradually returns to normal within six to twelve minutes. The heart rate always increases as the pressure rises, and rates can be reached as high as 255 per minute from an initial rate of 162. A similar hypertension and tachycardia results from doses of 0.1 to 1.0 mgm. of protoveratrine after the vagus has been cut, or after initial doses of the alkaloid have caused a simulated vagal block. Unlike the ester alkaloids the alkamine cevine even in doses of 40 mgm. does not have any characteristic vasopressor or cardioaccelerator action.

3. *The vasoconstrictor response.* Coincident with the increase in blood pressure caused by the ester alkaloids, veratridine or protoveratrine (154, 190), the blood flow in the femoral arteries increases. However, this is followed by a decrease of flow at the height of the blood pressure response; and throughout the blood pressure change the ratio of pressure to flow is increased, sometimes more than doubled, suggesting actual constriction. Vasoconstriction occurs in the acutely denervated limb and denervated femoral artery as well as in the innervated limb. At least two factors may therefore partake in this vasopressor response: 1, vasoconstriction by local action of the veratrum alkaloids upon the vessel wall, or vasoconstriction by substances (like epinephrine) released by the veratrum alkaloids and carried to the femoral bed by the blood stream; 2, an action of the veratrum alkaloids upon the nervous system leading to central vasomotor stimulation.

Vasoconstriction ascribed to local action of veratrine hydrochloride was observed by Kobert (148) in the isolated hindquarters of dogs perfused with dog's blood at 38°C., and in the isolated liver of the dog perfused from the hepatic artery. Concentrations of veratrine hydrochloride 1:10,000 were administered for 3 minutes into the leg arteries; 1:20,000 for 1 and 2 minutes and 1:40,000 for 5 minutes were given into the hepatic artery, causing a marked decrease in outflow from the organs which lasted from 9–15 minutes after the period of exposure. In the hind legs of mice and rats and in the isolated rabbit's ear perfused with Ringer solution, vasoconstriction with veratrine (151), although noticeable at lower concentration, was not constant below 1:20,000. In the hind legs of the frog (prepared according to P. Trendelenburg) veratrine 1:20,000 (151), germerine 1:40,000 (103) and protoveratrine 1:100,000 (103) caused vasoconstriction (see also section VI, A2, p. 411).

These observations on isolated organs cannot satisfactorily explain the hypertensive action of veratridine and protoveratrine in dogs described above, for the injection of as much as 0.5 mgm. of veratridine into the arterial supply from a "donor dog" to the perfused leg of a "recipient dog" fails to cause any constriction. On the other hand, the intravenous injection of 0.5 to 1.0 mgm. veratridine into the vagotomized "donor dog" causes an increase in "donor" arterial pressure with an initial flow increase in the perfused limb of the "recipient dog," but as the pressure in the "donor dog" reaches its maximum, flow rapidly decreases in the perfused limb of the "recipient dog." This indicates that a vasoconstrictor substance other than veratridine must reach the perfused femoral bed of the "recipient dog" from the "donor dog."

Conversely, if doses of 1 mgm. of veratridine or protoveratrine are injected into a vagotomized "recipient dog," blood flow in the femoral bed of a leg with nerves intact but perfused from a "donor dog" shows a long lasting increase. (This undoubtedly is in part caused by the reflex vasodilator effect of the alkaloids, but it may be in part due to a depressor reflex originating in pressoreceptor areas, not served by the vagus, and caused by the blood pressure increase in the "recipient dog" outside the perfused leg.) Under these conditions, when blood from the "recipient dog" cannot reach the perfused leg, there is no evidence of active vasoconstriction.

4. *The rôle of epinephrine.* The following facts, observed in the atropinized or vagotomized animal, suggest that epinephrine plays an important rôle in the pressor and cardioaccelerator effect of the veratrum alkaloids: the characteristics of the hypertensive response; the apparently humoral transmission by the blood stream of the vasoconstrictor effect, which, in the femoral bed of the dog, cannot be accounted for by the concentration of the veratrum alkaloids present; the characteristic increase in heart rate which cannot be due to a direct action of the veratrum alkaloids upon impulse generation in the heart (section V, E2, p. 406).

Direct measurements of epinephrine discharge into the suprarenal vein so far have been made only in a few cases in the dog, using the isolated gut of the rabbit to estimate the epinephrine concentration. While the available data are insufficient to allow of an accurate quantitative statement, they suggest that at the peak of the blood pressure rise after injection of 1 mgm. protoveratrine into the vagoto-

mized dog, concentrations of epinephrine of the order of 1:100,000 may be reached in the suprarenal vein (154).

The hypertensive effect of the veratrum alkaloids can be obtained by intravenous injections in the spinal cat as well as in intact animals (103, 182). It can be largely abolished by the extirpation of the suprarenal glands. Similarly, injection of the alkaloids into the aorta through the celiac artery of the eviscerated cat (154, 182) leads to the characteristic blood pressure and heart rate increase concomitant with contraction of the denervated nictitating membrane and inhibition of uterine activity. These effects can be prevented by removal of the suprarenal glands between successive injections. It was shown by Kusnetzow (161) that the isolated suprarenal gland perfused with Ringer solution releases epinephrine under the influence of veratrine.

The mobilization of epinephrine from the suprarenal gland is a property of the ester alkaloids. Cevine, the alkamine of veratridine, for instance, when injected into the aorta through the celiac artery in the spinal cat, proved ineffective in a dose 70 times larger than a strongly effective dose of its veratric acid ester (182).

While a direct effect of the veratrum alkaloids upon the suprarenal medulla leads to a release of epinephrine, the possibility remains that the veratrum alkaloids may act centrally upon sympathetic parts of the autonomic nervous system as well as upon certain parts of its sympathetic outflow, and that the action upon the suprarenal medulla represents one element only in a widespread sympathetic discharge.

5. *The possible rôle of the central nervous system.* A central vasomotor action was first considered by von Bezold and Hirt (18) when they observed blood pressure increase after veratrine in the vagotomized rabbit. In the cat with cold block of the vagus Jarisch and Richter (139) claim to obtain as a rule a blood pressure increase if 0.05 to 0.1 mgm. of veratrine hydrochloride is injected intravenously. The effect is still obtainable after denervation of the carotid sinus and removal of the thoracic part of the sympathetic outflow of the autonomic nervous system; but is absent or less pronounced after severing the cervical cord; and deepening ether anesthesia also decreases it. If a strong central vasomotor stimulation would be caused by large doses of veratridine or protoveratrine, this effect should become apparent in the femoral bed of a "recipient dog" perfused from a "donor dog," when one of the alkaloids is injected into the vagotomized "recipient dog." As mentioned above, under these conditions blood flow remains high in the perfused leg, and there is no evidence of vasoconstrictor impulses being carried from the vasomotor center to the vessels of the perfused leg by way of the femoral and sciatic nerves.

While there is no conclusive evidence in favor of a central vasomotor stimulation by large doses of veratrum alkaloids, a few experiments with small doses of veratrine suggest central vasomotor and sympathetic stimulation. When the receptor areas in the viscera of the chest are circumvented by injecting veratrine hydrocloride into a vertebral artery of the cat, doses between 0.01 and 0.05 mgm. cause a slight increase in blood pressure and acceleration of heart rate (136, 209). The suboccipital injection into the cisterna magna of 0.04 to 0.05 mgm. of

veratrine hydrochloride in the rabbit is followed within 3 to 5 minutes by fatal lung edema (141, 209), for which powerful central sympathetic stimulation is considered responsible. The rôle of epinephrine in these experiments was not ascertained. The phenomenon calls to mind the sudden lung edema in the rabbit caused by intravenous injections of epinephrine (171).

Kunishô (159) observed in rabbits a stimulation of uterine activity after the intravenous injection of 0.001 mgm. veratrine per kgm. This effect was abolished by sectioning the spinal cord between C_4 and C_5. After this, doses of 0.1 mgm. per kgm. were necessary to cause a second increase in uterine activity. Both these effects were not influenced by atropine but were entirely prevented by yohimbine. (The direct stimulating effect of veratrine upon the isolated uterus is not modified by yohimbine.) This is consistent with the assumption that epinephrine may be released and act as transmitter as a result of a central stimulating action as well as in consequence of an action upon ganglionic structures like the suprarenal gland. Further observations indicating possible central sympathetic stimulation are the inhibitory effect of small doses of veratrine upon the rabbit intestine in situ which can be prevented by severing the splanchnic nerves (160) and the hyperglycemic action of veratrine in the rabbit, which does not occur after cutting the cervical cord above the origin of the splanchnic nerves (220).

6. *Summary.* The veratrum alkaloids in high enough concentrations are vasoconstrictor by direct action upon the vessel wall. This is not likely to be of importance in the vasopressor response described above, observed with large doses when the vagus effect upon the heart is excluded. The available evidence indicates that epinephrine release plays an important rôle in the vasopressor and cardioaccelerator effect. Direct action of the veratrum alkaloids upon the suprarenal medulla causes a discharge of epinephrine. It is possible that in the intact animal a central sympathetic stimulation may also be involved in the effect upon the suprarenal gland. The epinephrine releasing action is a property of the ester alkaloids and probably not of the veratrum alkamines.

More extensive experimental work with the various pure veratrum alkaloids is required to establish the different sites of their action, especially in the central representation and in the sympathetic outflow of the autonomic nervous system.

E. *The Action of the Veratrum Alkaloids upon the Heart.* The elucidation of the chemical structure of the veratrum alkaloids (section I) has increased the interest in their action upon the heart. While the veratrum alkamines and cardiac aglycones are similar in their basic steroid structure, they differ in the nature of their additional rings. The unsaturated lactone ring at C_{17} of the steroid ring system of the cardiac glycosides appears to be intimately connected with their specific positive inotropic action. In place of this structure, veratrum alkamines have a nitrogen-containing ring system. This raises the question, as Craig and Jacobs (44) emphasize, "whether the cardiac action of both the cardiac glycosides and veratrine is not a property inherent in the sterol nucleus itself, once given the proper supporting groups in certain positions and the necessary stereochemical configurations."

The major effects of veratrum alkaloids on the heart are a positive inotropic effect, a moderate change in sinus rate, the production of irregularities, and a prolongation of the beat. The earliest definitive work in this field already described all but the first of these (18). The more striking cardiodecelerator effects produced by veratrum alkaloids in the intact animal are mediated by the central nervous system and have been discussed in section V, B. In the following paragraphs the actions of several veratrum alkaloids on the isolated heart will be discussed insofar as they resemble those of the cardiac glycosides. In section VI, A2, E and F the cardiac effects of these substances will be compared with the action of veratrine on nerve and muscle.

1. *The positive inotropic effect.* In the isolated heart of a marine snail, *Aplysia limacina*, L., Straub (243, 244) found that veratrine increases the maximal tension of the contraction. The same effect has been observed in the frog heart (146, 207, 246).

As is the case with other substances possessing positive inotropic action, this property can best be brought out in the hypodynamic heart. When this state was induced by perfusion of the frog heart with Ringer solution free of calcium, or by the use of citrate (which removes calcium), Ransom (204) and McCartney and Ransom (180) observed that veratrine caused an increase in the amplitude of the ventricular contraction. In the Straub heart veratrine completely abolished the impairment of activity due to calcium deficiency caused by Ringer solution containing $\frac{1}{3}$ of its normal calcium content (207). This positive inotropic action has been confirmed with pure alkaloids in isolated frog hearts perfused from the venous side, using as a measure of activity the restoration of the output to normal when it was decreased by perfusion with Clark solution containing only 50 per cent of the normal calcium content (154, 182). The concentration of protoveratrine or veratridine capable of restoring normal output without changing rate or producing irregularities was of the order of 1×10^{-7} while higher concentrations (2×10^{-4}) of the alkamine cevine were required.

In mammalian hearts the early observations of Hedbom (108) and Kuliabko (158) were more recently extended to the specific conduction tissue of dogs' and rabbits' hearts (90, 123, 253, 254). They were also confirmed in experiments on the heart-lung preparation of the dog, on the basis of suspension curves from the right ventricle (88). In each case veratrine produced a marked increase in the amplitude of contraction. As pointed out by Krayer and Mendez (152), the positive inotropic effect was not more carefully investigated prior to their own study for two reasons: 1, in the intact circulatory system of the mammal, the strong vagal action of veratrum alkaloids entirely obscures the direct cardiac action, and 2, even in the isolated heart the effect is not marked unless the heart shows signs of failure. This latter fact was clearly recognized by Hedbom, who stated, "Veratrin und Digitalin sind insofern von Interesse, dass sie zeigen, dass Herzpräparate, welche durch den Einfluss des Physostigmins (und vielleicht auch andere Schädlichkeiten) sehr schlecht arbeiten, durch diese beiden Gifte nochmals recht kräftig stimuliert werden können."

In the absence of a significant cardiac failure, Krayer and Mendez (152) saw

no consistent effect on systemic output, coronary flow, rate, mean arterial pressure, or pulmonary pressure in the heart-lung preparation of the dog with veratrine in doses up to 0.5 mgm. There was, however, a distinct though slight decrease in right and left auricular pressure; and an increase in blood supply, effected by raising the level of the blood in the inflow reservoir, was followed by an increase in auricular pressure which was less than that occurring before the administration of veratrine. When blood supply was kept constant, a marked decrease of heart volume (occurring without a significant change in heart rate or pulmonary arterial pressure) established clearly the inotropic nature of the veratrine action.

Like the cardiac glycosides, veratridine increases the efficiency of the failing mammalian heart while increasing its work performance and its oxygen consumption. The increase in efficiency is especially striking when the diastolic volume of the heart is kept constant (189).

Even severe failure in the isolated heart can be relieved and the work capacity of the heart restored to normal irrespective of whether failure is spontaneous or induced by pharmacological agents (barbituric acid derivatives, local anesthetics, atabrine). Under these conditions an effective dose of veratrine causes a marked increase in systemic output, a transient increase in coronary flow, a decrease in auricular pressure, and a very marked reduction in the size of the heart (152).

The interpretation of the change in diastolic volume in the failing heart is complicated by the action of veratrine on pulmonary arterial pressure and stroke volume. While the heart rate decreases usually not more than ten per cent, the stroke volume increases markedly as the total output rises. Pulmonary arterial pressure, changes of which have a strong influence upon the diastolic volume of the right ventricle, as a rule increases with the development of failure in the heart-lung preparation. This can be due to a change in arterial resistance as a result of alterations in the wall of the pulmonary vessels or it can be due to an elevation of left atrial pressure higher than normal pulmonary arterial pressure when the left ventricle becomes unable to empty. Veratrine causes a marked fall of this elevated pulmonary pressure. In a number of experiments with veratrine as well as veratridine (152, 191), the pulmonary arterial pressure increased for up to a minute and then fell markedly. Coincident with the rise in the pulmonary pressure, the systemic output rose and the right and left atrial pressures fell. Other things being equal, the rise of pulmonary pressure could be accounted for by the increased flow of blood through the lungs resulting from the increased cardiac output. The subsequent fall of pulmonary arterial pressure may be due to the fall of left atrial pressure, that is, to a decrease of back pressure. The further possibility remains, however, that a diminution of the resistance of the pulmonary circuit contributes to this fall of pulmonary arterial pressure.

The action of veratridine is qualitatively similar to that of veratrine and the pure alkaloid is only slightly more potent than the alkaloid mixture veratrine (191). Cevine, the alkamine of veratridine, still has the characteristic positive inotropic activity but has less than $\frac{1}{300}$ of the potency of veratridine. The minimal effective dose of cevine is 15 to 20 mgm. as compared to 0.05 mgm. of

veratridine under identical conditions in the heart-lung preparation of the dog with an average total blood volume of about 800 cc. The minimal effective dose of protoveratrine in producing the positive inotropic action is about 0.005 mgm. or only $\frac{1}{10}$ of that of veratridine (154). The degree of improvement of a failing heart attainable with protoveratrine, however, is less because of the narrower range between the minimal doses producing positive inotropic effects and those causing irregularities. The ratio of minimal irregularity dose to minimal positive inotropic dose is about 13 for veratridine, 5 for veratrine, and not more than 3 for protoveratrine.

The duration as well as the magnitude of the positive inotropic effect of veratridine (191) is similar to that of veratrine. An initial dose of 0.2 to 0.3 mgm., which in a failing heart restores normal output and normal auricular and pulmonary pressure, has a duration of action of 40 to 100 minutes. Smaller doses have a briefer action. The maximal effect does not appear abruptly but develops in a period of about five minutes. After large doses of cevine, however, the maximal improvement of output in the failing heart appears within one minute of the injection of veratridine (191). Repeated doses of veratrine (152) and veratridine (191) cause a progressively smaller and briefer effect. At this stage the effect can still be increased by increasing the dose, and relatively large doses, which given initially would cause irregularities, do not cause disturbances of rhythm but only lead to a transient improvement of output and work capacity. The moderate improvement in the work capacity of the failing heart with an initial dose of 0.005 mgm. of protoveratrine (154) lasts for about 15 minutes; with an initial dose of 0.015 mgm. it lasts for about 30 minutes. When subsequent doses of protoveratrine (not causing irregularities) have ceased to have a positive inotropic effect, veratridine is still effective.

2. *The effect upon spontaneity and conduction.* When the cardiac innervation and the adrenal medulla are excluded from affecting the heart, it is seen that veratrum alkaloids affect the beat of the heart by direct action in manifold ways. Bradycardia is usually the earliest effect, but sometimes tachycardia is seen. With larger doses, extrasystoles, bigeminy, pulsus alternans, auricular asystole, groups of rapid beats, persistent tachycardia, and ventricular fibrillation may occur. Auriculo-ventricular block and various kinds of intraventricular block are also found. A correspondingly diverse array of electrocardiographic tracings has been collected (see Boehm, 27, for older literature; 16, 27, 88, 89, 90, 123, 152, 154, 182, 191, 240, 241, 254, 266).

These cardiac irregularities appear to result from two types of changes produced by veratrum alkaloids in cardiac tissue, namely, changes in spontaneity, or tendency to produce spontaneous beats, and impairment of conduction of impulses, probably associated with alteration of the cycle of recovery of excitability after the passage of impulses. The diversity of the irregularities which have been reported depends upon the many combinations of site and degree of these effects in the different parts of the heart.

In the frog heart isolated according to Straub, the smallest active concentrations of protoveratrine, veratridine, and cevine cause only an increase in the

amplitude of the ventricular contractions. With higher concentrations, the heart rate decreases. In the isolated mammalian heart (152), initial doses of veratrine which do not produce irregularities have no constant effect on heart rate. In heart failure, especially when caused by barbituric acid derivatives, an initial dose of 0.2 to 0.3 mgm. as a rule produces a decrease of heart rate of about 10 per cent. Subsequent doses intensify this effect especially in the case of veratrine. Protoveratrine (154) causes no change of heart rate with doses which do not produce irregularities (0.005 to 0.015 mgm.). Even in the minimal positive inotropic dose (15 to 20 mgm.) cevine causes a definite slowing of the heart (191). After large doses of cevine which have caused a marked decrease in rate, veratridine, in doses which, given alone, do not cause a change in rate, produces a transient return of the heart rate to its original level (191).

The spontaneity of parts of the heart other than the sinus node is increased by somewhat higher doses of veratrum alkaloids. In inactive strips of mammalian auricle and turtle ventricle veratrine initiates persistent beating (216, 221). Ventricular extrasystoles of various sorts and the dominance of one or more ventricular pacemakers are evidences of an increase in the spontaneity of the ventricles seen in certain stages of the action of veratrum alkaloids. No similar increase in spontaneity is seen in veratrinized nerve or skeletal muscle.

Impairment of conduction commonly occurs with doses of veratrum alkaloids somewhat greater than those which simply change the sino-auricular spontaneity. The sudden appearance of partial A-V block in the veratrinized frog heart has been described repeatedly (see Boehm, 27, for references).

Sometimes intraventricular forms of block are the first to occur, leading to abnormalities of the ventricular complexes of the electrocardiogram and sometimes to pulsus alternans (156, 240, 241). Since increased spontaneity and failure of conduction coincide, it is not surprising that ventricular fibrillation often occurs.

De Boer (29) stated that veratrine lengthens the refractory period of the frog's ventricle. Drury and Love (52), however, maintained that de Boer was measuring not excitability but conduction. With a more precise method they showed that veratrine may shorten the refractory period of frog ventricle, but that simultaneously conduction may be severely impaired. In cardiac conduction tissue, on the other hand, Berk and Wachstein (16) showed a true lengthening of the refractory period. The conduction velocity of turtle ventricle, however, undergoes an initial increase before its marked decrease (89). Moreover, the threshold of the frog or turtle ventricle to induction shocks, like that of nerve and muscle (section VI, C) is first lowered and then elevated (48, 89). Hence it is not unlikely that the refractory period may be at an early stage shortened, and later lengthened. The data available at present do not illuminate this point.

In the isolated frog heart, sufficient doses of protoveratrine, veratridine, and cevine cause ventricular extrasystoles and auriculo-ventricular block. With high concentrations, systolic standstill occurs. Boehm (26) found this to take place within a few minutes with 1:550 cevadine. Systolic standstill is brought about with veratridine by a concentration of 2×10^{-5} (3×10^{-5}M) and with cevine by

1×10^{-2}M (182). With protoveratrine "Salzberger," Boehm (26) saw only diastolic standstill, whereas in the experiments of Krayer, Moe, and Mendez (154) protoveratrine "Jacobs" in concentrations of 1×10^{-3} to 1×10^{-4} caused ventricular standstill in systole within 5 minutes.

In the heart-lung preparation of the dog, initial doses of 0.5 to 1.0 mgm. of veratrine (152) or 0.65 mgm. veratridine (191) or repeated smaller doses cause arrhythmias and sudden tachycardia. With veratridine the heart rate may remain regular and tachycardia may occur with asystole of the auricle, suggesting A-V nodal or ventricular pacemaker. The establishment of permanent tachycardia is often preceded by short periods of acceleration. The severest effect is ventricular fibrillation. Protoveratrine (154) in doses of 0.02 mgm. usually provokes irregularities; auricular asystole and ventricular tachycardia are followed by ventricular fibrillation within 15 minutes after the administration. With large doses of cevine (191), periods of acceleration of the ventricles may occur, but even doses of 70 to 80 mgm. do not cause ventricular fibrillation.

In producing irregularities, veratridine is considerably more potent than is veratrine (191). For example, under comparable conditions, the administration of as much as 1.5 mgm. of veratrine in divided doses over a period of 80 minutes did not produce ventricular fibrillation, whereas this effect was brought about by 0.6 mgm. of veratridine given in three equal doses over a period of 50 minutes, and in another experiment by several doses totaling 0.75 mgm. given over a period of 90 minutes. Protoveratrine (154) is even more potent a cardiac poison than veratridine, their minimal irregularity doses being 0.65 mgm. for veratridine, and 0.015 mgm. for protoveratrine, a ratio of 43.

F. *The Complexity of the Circulatory Action of Large Doses of Veratrum Alkaloids in the Intact Animal.* The evidence contained in section V makes it understandable that in animals with the autonomic nervous system intact the analysis of the circulatory action of large doses of veratrum alkaloids presents great difficulties. The direct influence upon the rate, rhythm, and contractility of the heart, while absent or obscured with small doses, markedly modifies the circulatory action of large doses. Again, there are striking differences in this regard between the various pure alkaloids, as is illustrated by the great tendency to cause irregularities of rhythm which makes protoveratrine a substance so much more dangerous than veratridine.

It will be impossible, with substances of such varied pharmacological properties as the veratrum alkaloids possess, to analyze completely their circulatory action without a knowledge of the dosage ranges at which the influence upon other organ systems begins to affect the circulatory system and without knowing how this influence manifests itself. Reference to this was made when discussing the action upon the respiratory system. Relevant evidence for a fruitful discussion is lacking even in the case of the much investigated veratrine response of nerve and muscle.

VI. The ACTION OF THE VERATRUM ALKALOIDS UPON NERVE AND MUSCLE. The striking action of veratrum alkaloids on the skeletal neuromuscular system was first clearly described and analyzed in frog muscle by von Bezold and Hirt

(18) and Prévost (202). It has since been the subject of innumerable researches, most of them with veratrine (the mixture of veratrum alkaloids obtained from the seeds of *Schoenocaulon officinale*).

A. *The Repetitive Response.* For many years the opinion prevailed that only muscle was involved in the veratrine response and that veratrine had little or no effect on the nerve. The characteristics of the veratrine effect in muscle were therefore the first to be worked out, and only later was it shown that, except for the mechanical response, most of the phenomena seen in muscle could occur also in nerve.

1. *Skeletal muscle.* In order to understand the nature of the veratrine response in nerve and muscle, it is convenient to begin with the classical description of the phenomenon as seen in frog muscle (27). The resting muscle, treated with an appropriate dose of the drug, shows no obvious abnormality. If, however, a brief stimulus be delivered to the nerve or muscle, the usual quick contraction of the muscle is followed by a phase of slow relaxation which may last for more than twenty seconds. The myograms resulting from this type of response have varied shapes. Weak veratrinization leads to a twitch-like initial contraction and partial relaxation, followed by a slow secondary rise and fall. With stronger poisoning, the dip in the myogram diminishes, and the height of the secondary rise grows. With even stronger doses the curve presents a smooth rise to a maximum several times the normal twitch height, followed by a long, slow fall to the base line.

Since the completely curarized preparation, stimulated directly, gives typical veratrine responses, it may be concluded that the nerve is not necessary for this phenomenon and that the muscle alone is capable of producing it. The same conclusion may be drawn from the fact that denervated muscle exhibits the veratrine response for some time after the nerve has ceased to function (12, 74, 203).

It is generally agreed that the veratrine response begins with a twitch much like that of the normal muscle. The after-effects, however, have been the subject of much dissension. The main point to be settled is whether this part of the response is a tetanus or a contracture. It can be called a tetanus only if it consists of a succession of twitch-like responses, each one being conducted along the muscle fiber in which it originated, accompanied by a spike-like electric disturbance. If these conducted disturbances are absent, the veratrine response belongs to the heterogeneous group of contractures (83).

Until recent years, the prevailing view was that the slow phase of the veratrine response in muscle is a contracture. This was based on the experiments of all the early investigators purporting to show the absence of electric and mechanical evidence of conducted disturbances during this phase. If the response is to be classified as a tetanus, it must be accompanied by oscillatory electric disturbances; and all the early investigators agreed on the absence of these oscillations.

It is now evident that these experiments bore negative results because the electrical instruments then available were insensitive to the rapid disturbances of low amplitude which may occur in muscle. Thus the older investigators

recognized the tetanic nature of some conditions in which the motor unit, containing tens or hundreds of muscle fibers, fired with some degree of synchrony but were unable to detect the finer oscillations associated with the asynchronous firing of single fibers (cf. 50). The latter is the mode of the veratrine response insofar as it is a tetanic phenomenon (58). By skillful use of the string galvanometer, Paul Hoffmann (116) was able to demonstrate oscillations in the electromyogram of veratrinized frog muscle, particularly in the early part of the record, where the muscular volleys may be more or less synchronized. As the degree of veratrinization advanced, the frequency of the oscillations increased and their amplitude decreased. With full veratrinization, Hoffmann found a smooth electrogram. The string galvanometer records of others resemble more or less those of Hoffmann (28, 101, 143, 203).

With the cathode ray oscillograph, aided by electronic amplifiers, it has been shown conclusively that the veratrine response in muscle is under most circumstances a tetanus. This was first demonstrated by Bacq and Brown (12) in normal cat muscles stimulated directly or indirectly and after curarization or denervation. With weak or moderate degrees of veratrinization the conducted disturbances demonstrated in the electromyogram account fully for the mechanical record. In cats, fowl, frogs, and toads the two-peaked myogram of the weakly veratrinized muscle is accompanied by two corresponding bursts of spikes separated by a relatively silent period (58, 67, 106). In cats, Rosenblueth, Wills and Hoagland (219) observed no element of the mechanical response, even with severe veratrinization, which could not be attributed to the conducted disturbances demonstrated by the electromyogram. In frogs and toads, however, Feng (67) and Eichler (58) found, in confirmation of Hoffmann (116), that with severe poisoning the number and duration of oscillations conducted along the muscle fiber decreased despite the maintenance of tension.

If the veratrine response is to be classified as a tetanus, it must be propagated from a veratrinized to a non-veratrinized region of the muscle fiber, and hence the latter must show a veratrinic[3] response when the true veratrine response is set up in the veratrinized region. The amphibian M. sartorius is peculiarly adapted for this experiment because its muscle fibers pass in parallel arrangement almost the full length of the muscle. Hence when this muscle is fixed in the middle by gentle pressure and the two ends are made to record mechanically, it is possible to determine whether or not a response set up in one end of the muscle is propagated to the other end. If a part of one end of the muscle is poisoned with veratrine (with controls to show the absence of spreading of the alkaloidal mixture along the fibers) and brief stimuli are applied to the poisoned and unpoisoned ends, it will be apparent to what degree the veratrine response is propagated from the poisoned to the unpoisoned end of the muscle.

This double myogram experiment was performed in frogs by Biedermann (20) and Riesser and Richter (212) with a clear result. The veratrine response was localized in the half of the muscle, part of which had been treated with veratrine. In the unpoisoned half, only the initial twitch appeared. The older view, that the veratrine response is a contracture, rested on these results.

[3] For an explanation of the term veratrinic, see page 433.

The same experiment was performed in toads and frogs by Engelmann (61) and many others (65, 66, 67, 87, 121, 124, 179, 267) with the opposite result. All of these investigators showed that the veratrine response is, under most circumstances, propagated from the poisoned to the unpoisoned area of the sartorius. However, with high concentrations of the drug the propagation becomes less and less complete, until the result is not unlike that found by Biedermann (20). Using thermal measurements, and thus avoiding the depression of conduction which may occur at the fixed region of the sartorius in the double myogram, Feng (67) has shown that with moderate veratrinization the propagation from a poisoned to an unpoisoned region may be complete. Hence there could be no question of contracture.

Thus the electromyogram and the double myogram agree in the demonstration that the response of veratrinized muscle is largely if not entirely a tetanus. Only severe veratrinization of cold blooded muscles produces contracture.

Recent experiments of Kuffler (157) on single fibers from the frog's sartorius muscle suggest an explanation for the difference between contracture and tetanus in the veratrine response. If a single, brief stimulus is delivered to the veratrinized muscle fiber, a series of action potentials may be recorded from the stimulated spot. If, however, the recording electrodes are placed only a few millimeters away, it is found that some of the action potentials are absent. Some of the impulses originating at the stimulated spot have died out while being conducted along the fiber. Thus what is clearly a tetanus when recorded electrically at the stimulated spot in a single fiber would appear as partly tetanus and partly contracture when recorded from the whole muscle with the double myograph. "Contracture" may differ from tetanus in the veratrine response only by this failure of conduction. The block of conduction produced by large doses of veratrine will be further discussed in section VI, G.

2. *Various types of muscle.* A veratrine response occurs in all the vertebrate striated muscles in which it has been tested (1, 36, 74, 98, 107, 125, 147, 219). The phenomenon also occurs in certain invertebrate muscles (210). In other invertebrate muscles the typical myogram is absent. Riesser (210) ventures the generalization that the veratrine response occurs in muscles in which there is a tetanogenic system. Sereni (235) found cephalopod chromatophores to be a convenient single-cell preparation for the study of the veratrine response.

Strong solutions of veratrine have frequently been reported to produce a sustained shortening of excised striated muscle without the intervention of an external stimulus (31, 165, 242). In the hands of Lamm (163), who used the frog sartorius, this phenomenon occurred only with 10^{-2} veratrine. Bacq (11), using the rectus abdominis of the frog, found the same result with 10^{-4} veratrine.

In vertebrate smooth muscle systems veratrine usually affects the rhythm and amplitude of spontaneous mechanical activity when this is present. In some instances it produces a slowly developing, sustained shortening of the muscle, somewhat similar to what has been described above in skeletal muscle (10, 31, 51, 120, 232). Marfori (176) reported that the response of the frog's bladder to an electric stimulus after veratrine was longer than normal, with a slow relaxation; he considered this to be analogous to the veratrine response in striated

muscle. In the nictitating membrance of the cat (218), the phenomenon seen by Marfori occurs on delivering a single brief shock to the nerve supplying the membrane. If, however, the membrane is stimulated by epinephrine, veratrine does not increase the response. This result indicates that a veratrine response occurs in adrenergic postganglionic nerves but not in this smooth muscle. In view of the fact that veratrum alkaloids cause a release of epinephrine from the adrenal medulla (p. 401), it is possible that the abnormalities reported in veratrinized smooth muscle systems are due at least in part to the effects of veratrine on the neuronal elements of these tissues. The fact that veratrine sensitizes isolated intestinal strips from the rabbit or the vas deferens of the guinea pig to the stimulating effect of potassium ion (11) may have the same explanation. At present, however, it is impossible to exclude the existence of a veratrine response in smooth muscles (see also p. 401 and p. 402).

A number of observers have noted a prolongation of systole in veratrinized cardiac tissue (see Boehm 27, for older literature; 59, 182, 203, 221), occasionally to a marked degree, as in the heart of *Torpedo* (82). This prolonged contraction of cardiac muscle has generally been considered analogous to the veratrine response of skeletal muscle. Since the prolongation of contraction in skeletal muscle is due to a tetanic response and since tetanic contraction is generally agreed to be excluded by the properties of cardiac muscle, this interpretation is not warranted today. Gilson and Irvine-Jones (89) observed the prolongation of systole in the turtle's ventricle, and attributed it at least in part to the slowing of conduction, which was so marked that relaxation could be seen beginning at the proximal end of the strip while contraction was only beginning at the distal end. These effects of veratrum alkaloids on conduction velocity are much more striking in the heart than their effects in nerve and skeletal muscle (section VI, G).

Although the analogy between the contractions of veratrinized auricle or ventricle and those of skeletal muscle has been disappointing, the specialized conduction tissue of the mammalian ventricle has yielded suggestive results. These will be discussed in section VI, F.

3. *Nerve.* Von Bezold and Hirt (18) considered that veratrine prolonged the state of excitation of nerve in a manner analogous to what they saw in muscle. Their evidence, however, was uncertain, and a blast from Fick and Boehm (72) blew this opinion out of the minds of investigators for years.

Several attempts are made to test the effect on the muscular twitch of veratrine applied locally to the stimulated region of the motor nerve. Most of these were negative. In 1902, however, Ioteyko (122) found that some minutes after the application of veratrine to the nerve, a brief stimulus applied anywhere along the nerve produced a typical veratrinic response in the muscle. Wible (257, 258) repeated this experiment with the same result and demonstrated by a reversible nerve block between the veratrinized area and the muscle that it was indeed the veratrinized nerve which was producing the veratrinic response of the muscle.

These results imply that the stimulated area of veratrinized nerve gives off a

shower of nerve impulses. Many investigators had studied the electrogram of veratrinized nerve, however, without finding showers of spike potentials following the usual initial volley. Traces of oscillation appeared in the records of Bayliss, Cowan, and Scott (15) and Gasser, Richards, and Grundfest (86).

Clear demonstration of the repetitive response of veratrinized nerve to single brief stimuli was furnished in recent years (7, 53, 68, 70). Feng (68) comments on the probable reasons for previous failures to see this phenomenon.

In the amphibian and mammalian peripheral neuromuscular system the most sensitive indicator of the veratrine effect is the muscle (53, 70). With somewhat higher concentrations of the drug in the curarized preparation, repetitive responses may be demonstrated in the ventral root on applying a single shock to the intact nerve trunk; if the nerve is now cut close to the muscle, the repetitive discharges are found to be absent. With a still higher concentration of veratrine, the repetitive discharges arise in the veratrinized nerve trunk itself. Thus the intramuscular parts of the nerve fibers, that is, the nerve endings, are more sensitive to this effect of veratrine than the axons in the nerve trunk. The nerve endings are sensitive also to similar effects of guanidine and barium ion (54).

The repetitive response of veratrinized nerve is less striking in frogs than in cats, where the gradually diminishing discharge following a single shock may last almost half a minute and initially involve a large proportion of the nerve fibers in synchronous volleys at a rate as high as 660 per second (7).

The repetitive response to a single brief shock as a result of veratrinization has been observed in A, B, and C fibers of cats, A and C fibers of frogs, and in the nerves of certain invertebrates (7, 15, 53, 60, 86, 218). There is no reason to suppose that sensory axons are not affected as well as motor axons.

4. *Receptors.* Whether sensory receptors are subject to the same effect of veratrine as those described for nerve axons and muscle is difficult to judge from the available data. Sneezing and a burning sensation on contact with veratrine are properties of the drug recognized for many years. Since the circulatory reflexes (section V, A, B, C) arising as a result of the injection of veratrum alkaloids occur with very small doses, it seems unlikely that the nerve axons are sufficiently poisoned to respond repetitively. Yet the afferent discharge on injection of these drugs plays an important rôle in eliciting these reflexes. It is possible that the response of receptors to a given degree of stimulation may be rendered more intense by veratrine, thus producing a more frequent discharge in the afferent nerve.

5. *Synaptic and neuromuscular transmission.* The notion that veratrine may act on synaptic and neuromuscular transmission has cropped up repeatedly. Lapicque's theory of curarization was based partly on experiments done with veratrine. Since the drug can produce repetitive discharges in both nerve and muscle and, with higher doses, can block conduction in both of these tissues (section VI, G), the study of neuromuscular transmission is unusually difficult. No convincing evidence of curarization by veratrine is available.

Recent studies indicate that neuromuscular transmission in skeletal muscle is accomplished by the setting up of an adequate "end plate potential." This is

a relative negativity of the end plate region which may best be observed in partially curarized muscles on the arrival of a volley of impulses in the motor nerve. When it reaches a critical value, the all-or-none muscular response begins at the region of the end plate and is conducted over the muscle fiber. Physostigmine, curare and other drugs known to affect neuromuscular transmission change the size and duration of the end plate potential. Veratrine, however, does not significantly change this potential in the frog sartorius, even when repetitive nerve impulses arrive at the neuromuscular junction as a result of the veratrine response of the nerve (157). When an all-or-none response is initiated in the veratrinized muscle fiber at the peak of the end plate potential, its augmented negative afterpotential is merely added to the remnant of the normal end plate potential. There is evidence that the potential of the superior cervical ganglion which corresponds to the end plate potential of skeletal muscle is not affected by veratrine (55). Veratrine does not change the sensitivity of skeletal muscles to acetylcholine (37, 187). Thus, although both the nerve and the muscle may show evidences of veratrine response, the process of neuromuscular transmission appears to be unaffected.

6. *Summary.* Thus veratrine renders certain tissues capable of responding to brief stimuli in a characteristically prolonged manner. These responses we designate as veratrine responses. Instead of the normal single all-or-none impulse, the tissues exhibit a repetitive response consisting of a series of all-or-none impulses which long outlasts the stimulus. The prolonged veratrine response is therefore similar to the afterdischarge observed in some reflex systems.

The veratrine response is found in all varieties of nerve and skeletal muscle which have been tested. The phenomenon has been observed in the heart only in special circumstances. It occurs in a number of non-striated invertebrate muscles, but its occurrence in vertebrate smooth muscle has not been established.

B. *The Electrogram of Nerve and Muscle.* The features of the veratrine response so far considered are those which concern the overall function of nerve and muscle, namely, the origination and propagation of impulses and their mechanical effects. It is now appropriate to view some less obvious aspects of this phenomenon in an attempt to discover the mechanism of the abnormal behavior of the tissues involved.

1. *Demarcation potential.* If a nerve, a muscle, or the heart is injured at one point and a galvanometer is connected from this point to an intact region, the latter is found to be electrically positive to the injured point. This difference of potential is called the demarcation potential. It is generally agreed that the veratrine response can occur in nerve and muscle without change of the demarcation potential (7, 21, 157), although this potential may rise in one tissue (cat muscle, 219) and fall in another (cat sympathetic ganglion, 218) as a result of treatment with average doses of veratrine. High concentrations (10^{-5} and up) of the drug depress the demarcation potential (23, 102, 110); (frog heart, 112). The depression of demarcation potential will be discussed in section VI, G.

2. *Spike potential.* Using slow galvanometers, the earliest investigators of

the electrogram of veratrinized nerve observed a marked prolongation of the electrical response resulting from a single stimulus (18). With the capillary electrometer, Garten (81) first differentiated two electrical disturbances in the veratrinized olfactory nerve of the pike. Both were in the same direction, the intact lead going negative with respect to the crushed lead. First came a quick wave similar to that of the nonveratrinized nerve, and this was followed by a slow, prolonged negative wave. Hoffmann (116) found the same sequence in veratrinized frog muscle.

The first, quick wave of the electrogram of nerve and muscle is commonly referred to as the spike. It consistently accompanies the passage of conducted impulses in normal nerve and muscle.

Certain doses of veratrine increase the amplitude of the spike potential as seen in the electrogram. The increase is slight in nerve axons, but more marked in mammalian muscle, and even more marked in the superior cervical ganglion (7, 97, 218, 219). Its significance is not known. Larger doses in each case produce a decrease in spike magnitude. Evidence is presented that this decrease of spike magnitude occurs in the absence of block (7), although large doses of veratrine may produce a block of conduction in nerve (section VI, G).

In normal nerve a second maximal spike elicited within a short period after the first has the same amplitude as the first if it occurs any time after the relatively refractory period. After veratrine, however, a second maximal spike is smaller than the initial spike if it is delivered even much later than this period. If stimuli are applied repetitively at various frequencies, the spike magnitude is seen to reach a minimum 15 to 50 milliseconds after the initial spike. After this interval there is a gradual regrowth of spike magnitude, approaching the magnitude of the initial spike at a rate which is an inverse function of the frequency of stimulation (7). The same phenomenon occurs in cat muscle and superior cervical ganglion (218, 219). The time course of this phenomenon is similar to that of the repetitive response and the augmented negative afterpotential. This diminution of spike magnitude may be attributed partly to refractoriness of some of the axons as a result of repetitive activity and partly to the presence of a large negative afterpotential. Since in cat nerve it is different under anesthesia with different general anesthetics (214) other unknown factors probably also play a rôle.

3. *The augmented negative afterpotential.* The second wave of the veratrine electrogram is called the negative afterpotential. In the electrogram of normal nerve, a brief and relatively minor ripple following the spike is designated the negative afterpotential. Graham and Gasser (97) first clearly showed that veratrine produces a tremendous and long-lasting negative wave in place of this normal ripple. The veratrine afterpotential is not a propagated disturbance similar to the spike, but occurs only in the parts of the nerve or muscle which have been exposed to veratrine. An impulse originating in normal nerve, for example, has in this region a normal negative afterpotential, but when passing through a veratrinized region of the nerve, it leaves behind it the local, large, and prolonged negative afterpotential.

The augmented negative afterpotential is a constant and striking feature of the veratrine response. Its significance will be discussed in section VI, D. It has been found in all nerves so far studied (9, 15, 40, 81, 85, 97, 99, 100, 206, 218), in the mammalian sympathetic ganglia (167, 218), in vertebrate skeletal muscle (116, 219) and in the electric organ of *Torpedo* (82). The lack of effect of veratrine on the end plate potential of the skeletal neuromuscular junction and on the corresponding potential of the stellate ganglion has been noted above (p. 414).

The magnitude of the augmented negative afterpotential varies with the dose of veratrine and the tissue studied. In C nerve fibers it may approach the amplitude of the spike. It usually appears to be developing as the spike subsides, coming to a peak of amplitude some milliseconds later. In single muscle fibers from the frog's sartorius, Kuffler (157) finds that the interpolation of a spike during the negative afterpotential leads to a temporary annulment of the potential, which then grows again as a result of this second impulse. After a single stimulus, the negative afterpotential of nerve may last for seconds or even minutes. That of the normal nerve lasts but a few milliseconds.

Graham and Gasser (97) found protoveratrine to be less potent than veratrine in producing an augmented negative afterpotential in isolated frog nerve. The augmentation of the afterpotential was relatively slight. Its duration, however, was greater than that of the negative afterpotential of the veratrinized nerve—how much greater, these authors were unable to say because of the limitations of their recording system.

An augmented negative afterpotential may occur in veratrinized frog nerve without the tetanic veratrine response (e.g., 97, 157). Clear evidence of the occurrence of a tetanic veratrine response in veratrinized tissues without an augmented negative afterpotential is lacking. In single muscle fibers, Kuffler (157) found the augmented negative afterpotential with concentrations of veratrine insufficient to produce a tetanic response to a brief stimulus.

Within limits, the negative afterpotentials of successive impulses may be summed. This summation is evident when the stimulating electric shock evokes a series of impulses, i.e., the tetanic veratrine response. The second, third, and several subsequent impulses produce increments of the negative afterpotential begun by the initial impulse. There appears to be a limit to the amplitude of negative afterpotential which can be reached by the summation of effects of repeated impulses. Thus, beyond a certain frequency of maximal stimulation of a circulated mammalian nerve, no further increase of negative afterpotential occurs. Even a rapid rate of stimulation fails to prevent the gradual decline of the negative afterpotential (7). Conversely, the longer the interval between successive stimuli, the more does the electrogram resemble that obtained with the first stimulus. In cold-blooded nerve, it may be necessary to wait five minutes after the previous stimulus before a response of equal magnitude may be obtained (81).

The large, prolonged negative afterpotential of veratrinized nerve has usually been considered to be merely an exaggeration of the small, brief negative afterpotential of normal nerve. In fact, the occurrence or absence of an augmenta-

tion of a negative wave in the electrogram has been taken as evidence of the presence in various tissues of the basic mechanism underlying "the negative afterpotential." The negative afterpotentials of normal and of veratrinized nerve are sometimes considered to represent a slow phase of the recovery of the demarcation potential after the passage of the depolarization, or spike potential. Both are decreased by potassium and by catelectrotonus, which diminish the demarcation potential; and both are increased by calcium and anelectrotonus, which may augment the demarcation potential. Both may undergo summation as a result of tetanic stimulation. Both are more susceptible than the spike to cold and to anoxia (93, 98, 97, 229). The augmented negative afterpotential of veratrinized nerve differs, however, from that of normal nerve in having an early rising phase. For this reason, it cannot be attributed solely to a slowing of the reparative processes.

In view of the fact that the demarcation potential of nerve is maintained by oxidative mechanisms, the relation of the negative afterpotential to respiration is of interest. Schmitt and Gasser (229) found that with a degree of veratrinization which produced an augmented negative afterpotential (but probably no repetitive response), nerve volleys at 2 per second caused a larger increase of oxygen consumption than 120 volleys per second in the nonveratrinized nerve. Like that of the normal nerve, the negative afterpotential of the veratrinized nerve is much more sensitive to anoxia than is the spike potential. On readmission of oxygen it grows to a greater level than it had before the anoxia began. Thus oxidative metabolism is active during the veratrine negative afterpotential and essential to its maintenance. At least in the conditions when no repetitive response occurs, the metabolic cost per nerve impulse is much greater in the veratrinized nerve than in the normal. In skeletal muscle, however, when the tetanic veratrine response occurs, the efficiency of the external work performance is the same as that of a normal tetanic response (105). This contrast suggests that the metabolic abnormality of veratrinized tissues is marked in their excitation and conduction systems, and does not significantly involve the contractile system, whose activity accounts for the major part of the metabolism of muscle.

4. *Positive afterpotential.* So prominent a feature of the veratrine response is the negative afterpotential that the early positive afterpotential seen in normal nerve, if it still exists, is obscured. At the end of the long negative afterpotential of veratrinized nerve, however, there is a positive potential which slowly returns to normal (9, 85, 206). Furthermore, in two ganglionic structures, the superior cervical ganglion of the cat (167, 218) and the retina of the frog (247), at certain stages of veratrinization, one may see an augmented positive afterpotential not long after the end of the spike potential.

C. *Electrical Excitability.* Advancing degrees of veratrinization are generally agreed to produce first a rise and then a fall of resting electrical excitability. This has been shown for nerve, muscle, and the heart (7, 18, 48, 89, 97, 218). The changes of the parameters of excitation in frog muscle have been variously reported (37, 164). In circulated mammalian nerve the fall of excitability is

accompanied by the same shift of the conventional voltage-capacity curve upwards and to the left as is commonly seen when the excitability of a tissue diminishes; the rheobase rises and the time constant of excitation decreases (7).

In common with certain other conditions to be discussed below in section VI, M, veratrine changes the normal relation of closing anodal and closing cathodal thresholds. The anode stimulates at a lower voltage than the cathode, a reversal of the result in normal nerve (7).

The passage of a conducted impulse normally leaves in its wake a characteristic series of changes of electrical excitability. Of these, the absolutely refractory period and the succeeding relatively refractory period are, in frog nerve, little affected by veratrine short of doses which block conduction (97). A lengthening of the refractory period has been observed in crustacean nerve (60) (see also section V, E2).

Following the relatively refractory period, there frequently occurs a period of supernormal excitability. As a result of treatment with veratrine, this supernormality is often increased in intensity and markedly prolonged.

D. *Negative Afterpotential, Supernormal Excitability, and Repetitive Response.* There has been a tendency to attribute the main effect of veratrine on nerve or muscle to its augmentation of the negative afterpotential and to relegate the supernormality and the repetitive response to secondary positions as consequences of the augmented negative afterpotential. A fair degree of correlation has been established in non-veratrinized nerve between the afterpotential and the concurrent electrical excitability (see 63). Positive afterpotential tends to coincide with subnormal excitability, negative afterpotential with supernormal excitability.

The fact that veratrine enhances not only the negative afterpotential but also the supernormality has supported the thesis that negativity is a good index of increased excitability. The generally accepted fact that sometimes a large and prolonged negative afterpotential occurs in veratrinized nerve in the complete absence of supernormal excitability (7, 97) mars considerably the unqualified acceptability of this thesis. In the opinion of Graham and Gasser (97) this fact indicates that some other, unknown factor, in addition to the negative afterpotential, conditions the appearance of supernormality. Certainly a large negative afterpotential alone does not warrant the assumption of concurrent supernormality.

The repetitive response has been attributed to the excitatory influence of the negative afterpotential, or, in other words, to the supernormal excitability of which the negativity is considered to be the index (86, 157). The occurrence of a spike on the rising phase of the negative afterpotential is taken to be analogous to the occurrence of a spike at the peak of the endplate potential in skeletal muscle, or to the occurrence of a spike after the utilization time at the cathode when electric pulses are applied. Thus the negative afterpotential is interpreted as the sign of a local excitatory process which may reach liminal values and produce a conducted disturbance. In support of this thesis one may cite the fact that weak veratrinization produces a small increase of negative afterpotential

in single muscle fibers, but no repetition; whereas somewhat stronger veratrinization further increases the negative afterpotential and also produces repetition (157). Of the same import is the general correlation between the amplitude of negative afterpotential and the rate of repetitive response with various doses of veratrine (7), as well as the changes of frequency of repetitive responses at the beginning and toward the end of the negative afterpotential (53, 58, 67, 90).

The unqualified support of this view, too, is impeded by certain discrepancies. One is that the repetitive response may occur in the absence of supernormal excitability. Another is that with increasingly severe veratrinization the repetitive response may eventually decline while the negative afterpotential continues to grow more intense (7). Furthermore, in frog nerve painted with solutions of veratrine, repetitive responses to single, brief stimuli appear for a short time while the negative afterpotential is not very large, but disappear while the negative afterpotential is steadily growing. Finally, on aeration after anoxia, the negative afterpotential recovers to a value greater than that of the basal state, while repetitive activity remains depressed (68).

A general correlation certainly exists among these three aspects of the veratrine response, namely, negative afterpotential, supernormality, and repetitive response. The discrepancies noted above, however, indicate that other factors not now understood play a significant rôle. The physicochemical basis of the changes discussed here will be considered below in section VI, L.

E. *The Contractile System of Muscle.* The effects of veratrine in nerve and muscle so far discussed are related mainly to the processes of excitation and conduction. The prolongation of response to a brief stimulus is common to these two types of tissue, one with and the other without contractile powers. In both tissues, this prolonged response is attributed to a tetanic discharge of impulses. The other aspects of the veratrine response which will be described below are also common to the two tissues and hence to some extent independent of the contractile system. Yet the characteristic evidence of veratrinization has been the peculiar mechanogram of the veratrinized muscle.

Skeletal muscle is affected by veratrine in ways which are superficially similar to the positive inotropic action which it has on the heart (section V, E1). Veratrine increases the work done by a skeletal muscle in response to a single stimulus (see Boehm, 27). Yet the data do not permit the interpretation that this effect has the same mechanism in heart and skeletal muscle. Thus veratrine increases the work done by a skeletal muscle in response to a single stimulus by causing the response to be a tetanus rather than a single twitch. In the heart, the positive inotropic effect cannot be attributed to a tetanic response. Because of the tetanic response of veratrinized skeletal muscle, the comparison of the two tissues is difficult. In the heart, veratrum alkaloids produce an increased efficiency of the beat (189), whereas in skeletal muscle, veratrine does not change the efficiency of tetanic contractions (105). The available data are not sufficient to establish or exclude a positive inotropic effect of veratrine in skeletal muscle.

Generally speaking, the features of the veratrine response of skeletal muscle

are fully explained by the known effects of the drug on the excitatory and conduction systems of this tissue.

F. *Veratrine Response in Conduction Tissue of the Heart.* Although the analogy between the contractions of veratrinized auricle or ventricle and skeletal muscle has been disappointing, the specialized conduction tissue of the mammalian ventricle has yielded suggestive results. Goldenberg and Rothberger (90) found that in a certain stage of veratrinization, strips of the conduction bundle of the dog's ventricle have electrograms strongly reminiscent of those of veratrinized nerve and muscle. The initial negative deflection was followed by a prolonged negative wave. The frequency of spontaneous beating was very slow, and the long negative wave was followed by an isoelectric period before the next cycle began. Superimposed upon the negative wave in greater or less number were regularly spaced, sharp negative deflections similar in shape to the initial negative deflection but less in amplitude. The amplitude of these secondary negative waves increased as the interval from the initial deflection increased. The mechanogram showed a large contraction corresponding to the initial deflection, and a variable number of much smaller contractions corresponding to the later members of the series of secondary negative deflections.

In analogy with the results in veratrinized nerve and muscle, we may distinguish in these electrograms from specialized conduction tissue of the heart, the initial spike and the augmented and prolonged negative afterpotential as well as the repetitive spikes. The mechanogram shows that, although summation of contraction does not occur in the heart, a repetitive contraction occurs following the passage of an initial wave. In the increase of amplitude of the successive secondary spikes and contractions, we see the gradual recovery of the spike magnitude and, somewhat later, of the the contractile system. The abrupt cessation of secondary beats is similar to what is seen in the single fibers of veratrinized skeletal muscles (58, 67).

The prolonged negative wave of the electrogram of the veratrinized conduction tissue is similar to the negative afterpotential of nerve in being relatively more sensitive to anoxia than the initial and secondary spikes. Since with anoxia or cyanide poisoning, the negative afterpotential could be abolished without affecting the secondary spikes and contractions, whereas cooling abolished the secondary spikes and contractions but exaggerated the negative afterpotential, a certain independence between negative afterpotential and repetitive response exists in cardiac conduction tissue as in nerve.

When the calcium content of the fluid bathing the veratrinized conduction tissue was increased, the rate at which beats, or groups of beats, occurred was diminished, sometimes to zero, as in nonveratrinized conduction tissue. When a beat occurred under these circumstances, it initiated a long series of rapid discharges, characterized by repetitive spikes superimposed upon a long negative afterpotential and by mechanical responses corresponding to the spikes. This change in ionic environment seems to accentuate the similarity between the veratrine responses of conduction tissue and the nerve or muscle. In veratrin-

ized frog ventricles Seeman and Victoroff (233) found mechanograms and electrograms similar to those described by Goldenberg and Rothberger (90) in veratrinized conduction tissue when the calcium concentration was elevated.

In a subsequent paper (91) sodium arsenite was shown to produce responses of cardiac conduction tissue similar to those resulting from veratrinization.

G. *Conduction, and the Effects of Strong Veratrinization.* Weak or moderate veratrinization may slightly accelerate the conduction velocity in the heart (89). The main effect of veratrinization on conduction velocity in nerve as well as the heart, however, is depression, observed with more severe degrees of poisoning. When poisoning is severe, this is associated with a deterioration of other phenomena, namely, spike magnitude, negative afterpotential, repetitive activity, electrical excitability and demarcation potential (7, 27, 53, 67, 97).

Conduction is meanwhile blocked in an increasing number of fibers at various distances from the stimulating cathode. The probable influence of this factor on the earlier interpretation of the nature of the veratrine response in muscle has been discussed in section VI, A1. For many years the veratrine response of muscle was considered to be a special kind of local contraction, rather than a tetanus, because of experiments in which a failure of conduction of the veratrine component was a critical feature. In frog nerve, severely poisoned with veratrine, Graham and Gasser (97) found the negative afterpotential to diminish with distance from the stimulating cathode. This they attributed to a block of conduction of fibers at various distances from the cathode. It is interesting to note that after complete block of conduction and abolition of the spike by veratrine or other agents in the single axon of the lobster, a large local response still occurs (177).

In excised frog nerve, concentrations of veratrine from 1:200,000 to 1:50,000 produce a decrease in the demarcation potential whose extent is a function of the concentration of the alkaloid mixture. When the demarcation potential has fallen to a certain level, conduction fails. It may, however, be restored by an atmosphere containing 5 per cent carbon dioxide or by anodal polarization. Both of these agencies restore the demarcation potential and at the same time re-establish conduction (169). Under these circumstances, block of conduction would seem to depend upon a critical diminution of demarcation potential. Yet in circulated mammalian nerve, block may occur in the absence of a significant change of demarcation potential (7). The circulated nerve is, of course, continuously exposed to a tension of carbon dioxide similar to that exerted by an atmosphere containing 5 per cent of the gas. Some other mechanism of block would seem to predominate in these conditions.

In the heart the effects of veratrine on conduction are unusually prominent (section V, E2, p. 406).

H. *Stimulus, Initial Response and Veratrine Response.* In summary of the material so far covered it may be said that the effects of veratrine on nerve and skeletal muscle are in most respects the same. The effects of veratrine on the heart are in some ways similar to those on nerve and muscle. If the dose of

veratrine is not too large, a characteristic group of phenomena occurs when all-or-none responses are set up. With larger doses, additional phenomena arise, involving a failure of various features of cellular activity.

In moderately veratrinized, resting nerve or skeletal muscle no striking abnormality is evident. The oxygen uptake may be unaffected or slightly elevated (183, 229, 242) and the demarcation potential may be slightly raised or lowered. High concentrations of veratrine (1:1600 or more) poison certain oxidative systems in mammalian liver brei or slices (17).

Even when all-or-none responses are set up, the initial response is not strikingly different from the normal. It consists of a conducted disturbance characterized by a spike potential and, in muscle, a wave of contraction. The spike is often somewhat larger than normal, but the absolute and relative refractoriness of the fibers is not significantly changed (94, 97). The electrical excitability as tested with condenser discharges may be slightly elevated. The closing anodal threshold is significantly diminished and may be lower than the closing cathodal threshold.

Only after the initial response do the striking effects of veratrinization show themselves. The initial spike is followed by augmented negative afterpotential, prolonged supernormal excitability, and a long volley of additional spikes, the repetitive response, accompanied in skeletal muscle by tetanic contraction.

The veratrine response is therefore probably the result of conditions set up by the initial all-or-none response rather than a direct consequence of the applied stimulus. Support for this view might be drawn from the lack of effect of veratrine upon the summation of subliminal stimuli as tested by pairs of shocks (84, 95). Gasser (84) has shown, however, that when tested by single shocks after subliminal tetani, frog nerve shows a prolonged period of elevated excitability despite the absence of preceding all-or-none response. To this property of nerve he mainly attributed the enhancement by veratrine of the recruitment of nerve fibers (section VI, K). This effect of subliminal tetani upon excitability is similar to the action of veratrine on the supernormality which follows the spike. Nevertheless, the veratrine response occurs in a striking form when a single suprathreshold shock, or at the most two or three shocks, are applied. This action of veratrine on excitability therefore does not account for the veratrine response. This is borne out by the absence of effect on the veratrine response of electrical stimuli supramaximal with respect to the initial response (7). The veratrine response is a function of all-or-none responses and bears little direct relation to the stimulus.

I. *The Closing Tetanus and Accommodation.* Kodera and Brücke (149) were struck by the analogy between the slow response occurring in weakly veratrinized muscle with long shocks of many times rheobasic strength, and the "closing tetanus" observed in normal frog nerve or muscle with similar stimuli. By extrapolating from weak veratrine to no veratrine at all, they proposed that the drug acts on the mechanism responsible for the "closing tetanus," increasing its electrical excitability until the prolonged response becomes the predominant one, even when shocks of the usual strength and duration are used. The striking

aspect of the veratrine response, however, is its persistence long after the decay of the brief stimulus which elicited it. In contrast to this, the "closing tetanus" lasts little longer than the current which elicited it.

An effect of veratrine on the excitable mechanism of nerve responsible for the "closing tetanus" of the indirectly stimulated muscle is apparent from the work of Rosenblueth and del Pozo (217). They studied accommodation in circulated mammalian nerves stimulated by exponentially rising currents. In normal nerve they demonstrated a clear difference between the brief twitches produced by rapidly rising currents and the longer responses to slowly rising currents. As the rate of current rise of successive stimuli to the nerve was decreased (the voltage being increased so that the height of submaximal muscle response remained constant), the responses were quick twitches down to a certain rate of current rise, while beyond this rate they were slowly rising and prolonged. At the same point the curve relating the necessary voltage at the various rates of current rise has a break in its continuity. Nerve therefore contains two excitable entities, both involving conducted disturbances, one of which accommodates quickly and is associated with a brief twitch of the muscle, while the other accommodates slowly and is associated with a tetanic discharge lasting throughout the application of current. There can be little doubt that the latter, here studied in relation to slowly rising currents, is the excitability of the "closing tetanus," when studied with direct current pulses (see also 238).

Veratrine was found to change the accommodation of nerve chiefly with respect to this latter component, so that with a given rate of rise, much less peak voltage was necessary in order to produce a prolonged response (217).

In veratrinized muscle, as in nerve, a slowly accommodating response with relatively low threshold occurs. During the passage of a relatively weak constant current through veratrinized frog muscle, Meirowsky (181) observed a mechanical response with wave-like variations in intensity.

Since the prolonged veratrine response can occur on stimulation with brief shocks no stronger than those required to elicit single impulses in normal nerves and muscles, it is impossible to attribute the repetitive response to a failure to accommodate to a steady stimulus applied from outside. Conceivably, the augmented negative afterpotential resulting from the initial impulse might have the effect of an electrical current applied from outside. Against this possibility stands the relatively slight change in the excitability of the slowly accommodating mechanism (68, 217). Moreover, in the single muscle fiber, each repetitive spike briefly annuls the negative afterpotential of the previous spike before its own negative afterpotential begins to grow (157). Hence the negativity appears to be interrupted rather than sustained in the single fiber, and a reactivation seems more likely than a failure of accommodation. For these reasons it seems improbable that the change of accommodation produced by veratrine contributes to the production of the repetitive response.

J. *Factors Affecting the Veratrine Response.* Whatever they may be, the results of all-or-none response which are so strikingly affected by veratrine are less stable than the all-or-none response itself. Summation, fatigue, and the

influence of environmental changes affect the veratrine response to a greater degree than the all-or-none response.

1. *Summation.* Kodera and Brücke (149), confirmed by Hou (117), observed that weakly veratrinized frog muscle, when curarized, had two distinct excitability curves, depending on what type of response was taken as an end point. With stimuli of a certain duration applied successively at increasing strengths, the first response to be seen was a simple twitch as in the normal. Only when the strength was much greater did the slow veratrine response appear. Strength-duration curves plotted in the conventional manner for the veratrine response lay above and to the right of those constructed with the twitch as an end point. Indeed, the rheobase of the veratrine response might be as much as 100 times that of the twitch and the time constant of excitability of the former 20 times that of the latter. As the concentration of veratrine applied to the muscle was increased, there was little change in the twitch excitability, but that of the veratrine response increased with respect to strength as well as duration until the two excitabilities were indistinguishable, i.e., any effective shock elicited a veratrine response.

A very similar dichotomy of excitabilities was described by Bremer (33) in connection with his "neuromuscular contracture," namely, that single twitches occurred with shocks of relatively short duration, while long shocks produced veratrinic responses (although no veratrine was present). The necessary condition of Bremer's veratrinic "neuromuscular contracture" is stimulation of the frog nerve by two shocks separated by a brief interval. Since strong shocks of sufficient duration are known to set up repetitive firing in frog nerves at a sufficiently rapid rate, it seems likely that the prolonged responses of Kodera and Brücke (149) have the same initiating mechanism as Bremer's phenomenon. It has many times been demonstrated that with weak veratrinization a pair of stimuli delivered at a short interval may elicit a typical veratrine response, when one stimulus produces only a brief twitch. Thus the excitability curves plotted by Kodera and Brücke (149) for veratrine responses may represent the strength and duration of shocks which produce repetitive response in the nerve. The rôle of veratrine would then be to render the nerve self-reactivating. When the veratrinization is weak, a summation of the effects of two maximal stimuli may be necessary to demonstrate the self-reactivation.

2. *Fatigue.* When a single all-or-none response is elicited from a moderately veratrinized nerve or muscle, the augmented negative afterpotential immediately grows, supernormal excitability is demonstrable, and the volley of repetitive all-or-none responses follows, each additional response adding its veratrine effects to the previous ones. Whereas in the early part of this complex veratrine response a summation is apparent from a still-growing negative afterpotential and an acceleration of the repetitive spikes, very soon the response passes a maximum and gradually wanes. In single fibers (58, 67) and in the conduction tissue of the heart (90), the rate of repetitive activity diminishes slightly and then suddenly ceases. In multi-fibered nerves or muscles, the waning of the veratrine response may last for thirty seconds or more, and full recovery may not occur for as long

as five minutes. Thus the veratrine response, while at the beginning seemingly self-perpetuating, is eventually self-limiting.

If additional stimuli are applied before recovery is complete, the resulting initial response is normal soon after the relatively refractory period, although for some time spike magnitude may be decreased (section VI, B2). The veratrine response is, however, more or less intensified.

For stimuli applied after the maximum of the veratrine response, the later they are applied, the greater is the intensification of the veratrine response. If stimuli are applied regularly at suitable intervals, this intensification is progressively lessened. Thus the veratrine component of the response to each successive shock is diminished, until, at moderate rates of stimulation, it may after a few shocks entirely disappear. The normal all-or-none spike or twitch remains, without augmented negative afterpotential, supernormality, or repetitive response. Thus, while the all-or-none responses to electrical stimuli remain normal, the veratrine component has fatigued.

3. *Other Factors.* An instability of the veratrine response as compared to that of the all-or-none response is observed also when the physical or chemical environment of the tissue is changed. The negative afterpotential, the mechanical veratrine response of muscle, and the repetitive response of nerve are more sensitive than the spike or twitch to cold, to anoxia (68, 97, 162, 187), and to carbon monoxide (229). The recovery of negative afterpotential after cold is slower than that of the spike; and after anoxia or during illumination of nerves treated with carbon monoxide, the negative afterpotential, unlike the spike, rises temporarily to a higher level than before the poisoning.

Changes in the common inorganic ions affect the veratrine response to a greater degree than the all-or-none response. A comparison of the effects of veratrine with those of the common inorganic ions shows that the effects of veratrine cannot be due to a simple imitation or exaggeration of the effects of any one of these. Yet there are important relations between some of these ions and veratrine. For example, the effects of veratrine are sensitive to changes in calcium concentration of the perfusing or bathing fluid. When lack of calcium has weakened the beat of the frog heart, veratrum alkaloids restore the beat to normal (154, 180, 182, 204, 207) (section V, E1). On the other hand, lack of calcium favors the production of cardiac irregularities by veratrine (207). Thus in the heart with respect to contraction, calcium and veratrine are synergists, but with respect to excitation and conduction, they are antagonists.

In nerve and muscle the antagonism of calcium to most of the effects of veratrine is marked. The dose of veratrine necessary to produce a minimal veratrine response in excised frog muscle is a continuous function of the calcium content of the bathing fluid. A change of calcium chloride content from 0.1 to 0.5 per cent multiplies by ten the minimal dose of veratrine (53, 68, 163, 187).

This antagonism recalls the generalization that calcium stabilizes biological membranes. Calcium lack may be associated with spontaneous discharge of nerve or muscle impulses and a marked diminution of accommodation. With veratrine, spontaneous discharge is absent and the accommodation is not

markedly changed. Lack of calcium does, however, sometimes lead to responses somewhat reminiscent of the veratrine response (162). Calcium opposes the diminution of demarcation potential produced by veratrine or potassium (102). It also opposes the excitatory effect of potassium in frog muscle when this is favored by veratrine (11, 107). Calcium counteracts the paralyzing action of strong concentrations of veratrine on the frog nerve-muscle preparation (65). Citing experiments on dogs Bacq and Goffart (14) advocated the use of calcium salts together with magnesium salts for combatting the toxic effects of veratrum alkaloids.

In some respects the antagonism of calcium to veratrine in nerve is replaced by a similarity of effects. A high calcium content of the bathing fluid favors the appearance of recruitment in nerve fibers (84, 255). High calcium concentration also enhances the negative afterpotential and lengthens the supernormal period of frog nerve (93). Veratrine produces these same effects, but to a greater degree (section VI, B3 and C).

The relations of veratrine with potassium are as tangled as those with calcium. The dose of veratrine necessary to produce a minimal veratrine response on electrical stimulation in frog muscle increases with the potassium content of the bathing fluid (187, 248, see also Boehm, 27). Potassium also diminishes the negative afterpotential and repetitive activity of the conduction tissue of the dog's heart (90). On the other hand, veratrine sensitizes smooth and striated muscles, nerve, and probably also certain receptors to the stimulating action of potassium (8, 11, 107, 188, 215, 237). With respect to this action in muscle calcium antagonizes the effects of veratrine, i.e., desensitizes the muscle to potassium. The theoretical importance of the sensitization to potassium will be discussed in section VI, L.

Magnesium and strontium, like calcium, oppose the effects of veratrine (11, 102, 134, 184, 185, 186, 248). Barium, however, may cause repetitive responses somewhat similar to veratrine responses (54, 70, 91, 248), and veratrine sensitizes certain responses to barium and to rubidium, as to potassium (8, 11).

Quinine is antagonistic to veratrine in several ways. It decreases the mechanical responses and the repetitive spikes of veratrinized muscle. When larger doses of veratrine are used, quinine opposes the block of conduction which ensues. Whereas stimulation of non-veratrinized muscle by potassium is relatively unaffected by quinine, the sensitization of muscle to potassium by veratrine is impeded by quinine (106, 187, 188, 248). In these relations to veratrine it resembles calcium.

Quinine possesses a rather general action against repetitive responses. While enhancing twitch tension and the spike potential in normal muscle, it diminishes the tension of tetani and the corresponding spikes. The repetitive responses of physostigminized muscle stimulated indirectly are abolished (106). Fibrillation in the heart is opposed. Several muscular phenomena similar to the veratrine response are also antagonized by quinine, namely, Bremer's "neuromuscular contracture" (34), myotonia of man and goats (144, 150), and the veratrinic responses to 9-phenanthroate and dihydronaphthacridine carbonic acid (3) (section VI, M).

A diverse group of drugs which has been reported to antagonize veratrine effects in muscle need only be listed: cholesterol (173); atropine (107, 168, 211, 248); pilocarpine (168); santonin (248); camphor (231, 250); cocaine and procaine (211, 231); salicylate, and tyrosine ester (230). According to Machens (173) lecithin enhances the action of veratrine in muscle.

K. *Interaction, Recruitment, and the Two-Peaked Myogram.* The responses of nerve and muscle fibers after conduction are normally all-or-none in character, and there is valid evidence that each fiber is normally insulated from its neighbor in nerve trunks and muscles, so that each impulse passes to the end of its individual fiber without stimulating other fibers. Accordingly the height of contraction of a muscle in a single twitch has been generally considered to be rather strictly a function of the number of fibers stimulated. The same laws cannot be applied to the veratrine response of frog muscle (18, 162, 192). With stimuli of increasing strength, from threshold to maximal, applied to curarized and veratrinized muscle, the rate of increase of the early, twitch-like part of the mechanogram is much like the normal; but that of the late, prolonged part follows a different course. Thus the response to a slightly supraliminal stimulus consists of a relatively marked twitch-like phase and hardly any prolonged contraction; but with increasingly stronger stimuli the proportion between the prolonged contraction and the twitch grows steadily. If we take the height of the twitch-like phase to represent the number of fibers stimulated, then the amount of prolonged contraction per stimulated fiber increases with the number of fibers stimulated (see also Querido, 203).

In 1931, Wyss (265) suggested that under the influence of veratrine the impulses in muscle fibers are transmitted to adjacent fibers. This mechanism would explain the phenomenon described above if the excitation of neighboring fibers is subject to summation. In this case a few scattered fibers activated by the stimulus would produce little effect on unstimulated fibers and hence little after effect, but with a greater density of initially active fibers the effect on neighboring fibers would be sufficient to excite some of these. These additional impulses would augment the excitatory influence of the initially active group on other fibers not yet rendered active. Herman (111) tested this theory by eliciting twitches of identical submaximal height in veratrinized muscle with stimuli from localized and from diffuse electrodes. The twitch from diffuse electrodes was followed by a longer-lasting and greater veratrine component than that from localized electrodes. If all the fibers within a certain radius from the localized cathode were excited in the latter case, only a few fibers beyond the periphery of this field would be available for the excitatory effect of adjacent active fibers. If, on the other hand, an adequate density of initially active fibers were interspersed with inactive ones, the conditions would be favorable for stimulation of the inactive fibers by adjacent, active ones.

Whereas the theory of interaction among muscle fibers remains in this speculative plane, interaction among nerve axons is supported by clear experimental results. Feng and Li (69) showed that veratrine may cause the activity of some fibers of a nerve trunk to initiate activity in adjacent fibers. They stimulated one ventral root (A) and recorded from another (B), both having been cut cen-

trally and the nerve trunk into which they both led having been cut peripherally. Without veratrine, stimulation at A produced no response from the fibers of B; application of veratrine to the common nerve trunk led to the appearance of impulses at B, 4 to 30 milliseconds after stimulation at A.

Veratrine enhances and prolongs changes favoring interaction among fibers observed in non-veratrinized nerve (214). If the excitability of a zone of normal nerve trunk is raised by one of several methods, the passage of impulses along some of these fibers induces activity in other fibers at this zone. The new impulses arise either during the rising phase of the spike potential, or after the spike potential (see 178, 214, for references). If veratrine is present, the passage of impulses in the conditioning fibers induces such marked excitation in the test fibers that new impulses arise in the latter.

A phenomenon resembling interaction, which is similarly enhanced by veratrine, is what Gasser (84) designated as recruitment of nerve fibers. If a nerve trunk is stimulated with submaximal electric shocks at a relatively high frequency, the number of nerve fibers responding to the stimuli gradually increases as more and more previously inactive fibers are recruited. Factors which favor the negative afterpotential also favor this phenomenon. After veratrinization, a remarkable degree of recruitment occurs with notably lower frequencies of stimulation than in normal nerve. Gasser attributed this mainly to a lengthened period of summation of subliminal stimuli, which he demonstrated to be present by testing the excitability of the nerve after a subliminal tetanus. Rosenblueth (215), however, showed that recruitment occurs independently of the applied electrical stimuli. He therefore attributed recruitment mainly to the influence of responses in the lower-threshold fibers on their not-yet-recruited neighbors, that is, to interaction among nerve fibers.

The effect of veratrine upon the interaction among fibers, if it applies as well to muscle fibers as it does to nerve fibers, suggests an explanation for the two-peaked mechanogram of veratrinized frog muscle. When the dose of veratrine is relatively small, a single maximal stimulus to the nerve or muscle elicits a twitch-like initial contraction and partial relaxation, followed by a slow secondary rise and fall (see Boehm, 27). The electrogram of a multifibered muscle in this condition shows a pause between the synchronous initial spike and the repetitive discharge of some of the fibers (58).

If only a few fibers are sufficiently veratrinized to respond repetitively when a single impulse is elicited in them, the first repetitive response of these may be expected to add its excitatory effect to whatever subliminal excitatory state remains in the fibers which did not respond repetitively. Some of the latter may be expected to respond to this summation with all-or-none responses; and the excitatory effects of these would affect still other previously non-repetitive fibers. Thus by the interaction and summation peculiar to veratrinized tissue, a progressively greater fraction of the muscle would become involved in an intense veratrine response. Hence the original maximal twitch would give way, first to a partial relaxation because at the beginning only a few fibers fire repetitively, but then to a progressive increase of contraction as more and more fibers are recruited.

The repetitive response so characteristic of veratrinized nerve and muscle may result from the same conditions that render the interaction among nerve fibers so prominent. Since responses in some fibers of a veratrinized nerve trunk can so markedly affect other fibers as to elicit new responses from them, the setting up by the initial response of additional responses in the same fiber should also occur. For this it is necessary that the excitatory state resulting from the initial response should outlast the refractory period, and should be capable of acting on the same fiber at least as readily as it does on neighboring fibers. Since even in non-veratrinized nerve a slight excitatory interaction is present which outlasts the refractory period of the conditioning fiber, the first of these conditions is probably satisfied. The distance of even the closest neighboring fibers should attenuate the excitatory influence; and hence the conditioning fiber itself, if susceptible, should be more strongly affected than its neighbors.

Since the possibility exists that repetition and interaction depend on the same excitatory effect of the initial response in veratrinized nerve and muscle, any clue to the mechanism of interaction may provide solutions to many aspects of the veratrine problem.

The rôle of the electric potentials of the conditioning fibers in causing excitation of their neighboring fibers is difficult to analyze in veratrinized nerve. Recruitment and interaction occur in nerves which show more or less repetitive responses to single shocks and more or less of the augmented negative afterpotential. It is generally agreed that in non-veratrinized nerve the electrotonic effects of the spike potential of some fibers on their neighbors is responsible for a rise and a subsequent fall of excitability of the latter. The initial spike has the same effect in veratrinized nerve (215). In the repetitive response, however, many spikes occur in the active fibers asynchronously for a long period. That the excitatory state set up by these spikes in neighboring fibers may play some rôle in interaction is suggested by the fact that interaction is poor when a diminution of spike amplitude accompanies the veratrine response (214). A series of spikes in one fiber might be expected to have the same effect on a nearby fiber as a subliminal tetanus, and hence lead in the veratrinized nerve to the long period of increased excitability which Gasser (84) detected in these conditions.

The explanation of the late phase of interaction in nonveratrinized nerves is controversial. Marrazzi and Lorente de Nó (178) attributed it to prolonged electrotonic effects similar to those briefly described by Lorente de Nó and Davis (170) after the application of direct current. In the veratrinized nerve, the abnormal development of the negative afterpotential contributes to the difficulties of analysis of interaction. Gasser (84) noted that the factors known to augment the negative afterpotential—among them, veratrine being the most prominent—favored the appearance of the phenomenon of recruitment. But interaction in veratrinized nerve may be poor when the negative afterpotential is pronounced; and vice versa, it may be pronounced when the negative afterpotential is only slightly greater than that of normal nerve. Furthermore, in non-veratrinized nerve the late phase of interaction does not follow the course of the afterpotentials and is as great as that occurring during the spike, despite the small amplitude of the afterpotentials in these conditions (214). These

discrepancies impelled Rosenblueth (214) to dismiss the theory that the late phase of interaction depends upon changes of the inactive fibers resulting from the electrical potentials of the active fibers. He proposed that the late interaction is due to a chemical substance, released from the active fibers as the impulses pass by and diffusing to the inactive fibers, there to act in an excitatory manner.

In another field, that of neuro-effector transmission, considerable advance resulted when Otto Loewi conceived the simple but effective experiment of determining the effect of the products of stimulation of the nerves of a donor heart by perfusing the fluid from this heart through a test heart. Perhaps the same contribution to the problem of interaction between veratrinized fibers has been made by Szent-Györgyi, Bacq, and Goffart (245). They perfused the veratrinized hind legs of a frog and led the perfusate through the veratrinized hind legs of another frog. When the muscles of the donor frog were stimulated, they responded in the typical veratrine manner. And when the fluid which was perfusing the donor preparation during its veratrine response reached the test preparation the latter also showed muscular contraction. Curarization did not interfere with the phenomenon. Miyake (188) has confirmed these results. Hence activity of the veratrinized donor muscles imparted to the perfusion fluid the power of exciting activity in the veratrinized test muscles. Here the electrical field of the donor muscles cannot act on the test muscles. The exciting influence, whether released into the perfusing fluid from the active muscles, or by their activity unmasked, must be a chemical change carried by the perfusion stream. And since chemical interaction is effective in this way between distant muscle fibers, it can also be effective between nearby fibers.

L. *Theories of the Veratrine Response.* Enough has been stated to make it plain that the veratrine response is a result of conditions set up by the initial all-or-none response of muscle and nerve. The literature as to the theory of the veratrine effect is devoted mainly to the nature of these conditions.

The theories that the veratrine effect is due to some increase of or sensitization to the potentials developed in nerve and muscle as a result of the all-or-none response have been discussed in section VI, D and K. Veratrine does affect markedly the negative after-potentials in nerve and muscle and also increases the excitability of these elements to electric current. Despite the discrepant experimental results listed in section VI, D, these electrical theories cannot at present be dismissed. They do not attempt, however, to analyze the physicochemical mechanism by which veratrine renders abnormal these potentials or this excitability.

The chemical theories of the veratrine response suppose that all-or-none responses of veratrinized nerves or muscles cause chemical changes in the cellular environment which are responsible for the peculiarities of the veratrine response. One such theory is that of von Frey (78) and Lamm (162), who proposed that veratrine is itself changed as a result of the all-or-none response of muscle into a substance which produces further contraction. In support of this view Lamm cited two experiments. In one, he mixed a quantity of veratrine with chopped muscle and then extracted the muscle with ether. When he redissolved the

ether extract and assayed it on the frog sartorius, he recovered more veratrine activity than was accounted for by the quantity of veratrine originally added to the muscle. The result of this experiment needs confirmation using pure veratrum alkaloids. In the other experiment, Lamm immersed a succession of muscles in Ringer's solution containing originally a certain concentration of veratrine and measured for each muscle the time of immersion before a minimal veratrine response occurred on electrical stimulation. The second, third, and fourth muscles required successively briefer immersion before the veratrine effect was evident. The later muscles of the series required successively longer immersion until finally no veratrine response could be elicited. Lamm's interpretation of these results was that contact of the veratrine with muscles changed the veratrine into a more potent form, at the same time fixing some of it in the muscle substance.

Verzár and Felter (252) could not accept the theory of von Frey and Lamm that veratrine was chemically changed by the muscle because so many substances of diverse chemical nature have the same effect as veratrine. As an alternative, they suggested that veratrine and these other substances render the muscle more permeable to a stimulating substance released as a result of the twitch. They were aware of the supernormal period of non-veratrinized tissues and were actually proposing that supernormality is the result of this stimulating substance. Fatigue of the veratrine effect would occur, according to their view, when the stimulating substance has penetrated into the muscle cells. They had nothing to say about the chemical nature of this stimulating substance.

The experiment of Szent-Györgyi, Bacq and Goffart (245) described on p. 430 suggested the possibility of identifying a stimulating substance released from muscle during the veratrine response. Since the stimulating substance has its effect on the test frog muscles even after complete curarization (13), it cannot be acetylcholine, whose stimulating effect on muscle is abolished by curare. Moreover, acetylcholine from the test muscle would be largely destroyed in the course of the perfusion by the tissue cholinesterase. In certain concentrations veratridine and veratrine have anti-cholinesterase activity (9, 193). Veratrine does not, however, change the threshold of muscle to acetylcholine (37, 187).

Since the responses of the test muscles resembled those seen when the potassium ion content of the perfusion fluid is elevated, the behavior of potassium was investigated (13). It was found that the potassium content of the perfusion fluid coming from the donor muscles during muscular activity was enough to stimulate the muscles of the veratrinized test frog. If fresh perfusion fluid whose potassium content had been elevated to the level found in the perfusate of the stimulated donor muscles was perfused through veratrinized test muscles, responses were produced in the latter which matched those seen in the experiment of Szent-Györgyi, Bacq, and Goffart (245). Veratrinization of the donor muscles increased only slightly the potassium content of the perfusate from unstimulated or stimulated muscles. The main rôle of veratrine was the sensitization of the test muscles to potassium (section VI, J3). Thus tetanic muscular activity

can release enough potassium ion to stimulate a distant veratrinized muscle even after dilution in the perfusion fluid.

On the basis of these results Bacq (11) proposed that potassium ion is the stimulating substance released by the all-or-none response of veratrinized muscle which accounts for the veratrine response. When applied to muscle in high enough concentration, potassium ion elicits all-or-none responses. Denervation and curarization do not interfere with these effects. During contraction muscle loses some of its abundant intracellular potassium in exchange for sodium (71). Bacq supposed that the potassium liberated at the surface of the normal muscle cell as a result of the twitch is quickly rendered incapable of restimulating the muscle cell. Veratrine would then impede the inactivation of potassium and hence permit it to produce a second response from the muscle. This response would of course release more potassium, which would then intensify the restimulating effect. Thus veratrine would render the muscle self-reactivating by making the potassium released with the initial twitch an effective stimulating agent.

In investigating the mechanism of the late phase of interaction among nerve fibers, Rosenblueth (214) considered the possibility that potassium released from the conditioning fiber might change the excitability of the tested fiber. Potassium ion injected into the circulation of the nerve favors interaction. This action is accentuated by veratrine, and veratrine sensitizes the nerve to this action of potassium. Rosenblueth therefore tentatively proposed that the late phase of interaction between nerve fibers is due to the diffusion of potassium from the active fibers to nearby inactive ones. The enhancement of interaction by veratrine might then be due partly to the repetitive activity of the conditioning fibers and partly to the sensitization of the test fibers to potassium.

The potassium theory of the veratrine response should be considered at present as no more than a working hypothesis. According to the argument of Bacq (11) it would account for repetitive response to single, brief stimuli. Rosenblueth's (214) experiments make it a plausible explanation of interaction. Yet recruitment, a phenomenon closely related to interaction in at least some respects, is opposed by potassium and enhanced by calcium (84, 255). Moreover, potassium in concentrations near that of physiological fluids opposes the action of veratrine. Since potassium decreases the demarcation potential, it is conceivable that potassium remaining in relatively high concentrations outside the nerve, as supposed in Bacq's theory, might account for the negative afterpotential. The depressing effect of potassium on the spike potential might also account for the diminution of spike amplitude observed after the initial spike in the veratrine response. Yet increasing the potassium content of the bathing fluid decreases the negative afterpotential of non-veratrinized nerve (93). The potassium theory also fails to account for the increased metabolism of the period of afterpotentials demonstrated in nerve by Schmitt and Gasser (229). Guanidine and aconitine also sensitize muscle to potassium (11, 107); yet their action is not identical with that of veratrine.

M. *Veratrinic Responses.* The effects of veratrine on the frog's gastrocnemius

are so striking and so easy to demonstrate that the rôle of this particular mixture of alkaloids in bringing about the characteristic response is apt to be overemphasized. There are many other ways of producing responses similar to the veratrine response. Since responses which resemble those produced by muscarine or nicotine are called muscarinic or nicotinic, it seems proper to designate those which resemble the veratrine response as veratrinic.

Most of the veratrinic responses have been less completely analyzed than the veratrine response itself. Hence the concepts developed for the latter have been applied to the former. In general these veratrinic responses have been interpreted as contractures on the basis of the same sort of evidence which led to the opinion that the veratrine response is a contracture. In a few instances this interpretation has been revised on the same basis which led to the revision of the opinion on the veratrine response. In others the crucial experiments have not been performed.

A group of veratrinic responses has been shown to occur in the absence of any pharmacological agent. Occasionally otherwise normal amphibian muscles have been reported to respond to single slightly supramaximal induction shocks with a veratrinic myogram (31) which, like the veratrine myogram itself, diminishes with repeated stimulation until it resembles the normal twitch. The variables which favor the appearance of this phenomenon in some frogs and not in others are little understood.

Bremer (33) has studied another such phenomenon, which he called "neuromuscular contracture." It occurs in a certain proportion of frogs or toads. The critical circumstance for its production is the arrival at the muscle, at an interval of about 3 milliseconds, of two stimuli. The veratrinic response occurs on stimulation via the nerve or on direct stimulation of the normal or curarized muscle. Bremer was unable to find oscillatory action potentials accompanying the response and hence called the phenomenon a contracture. His apparatus, however, was too insensitive to record fibrillary activity of the muscle. The rôle of summation and fatigue in Bremer's phenomenon is similar to its rôle in the veratrine response. Quinine opposes Bremer's phenomenon, as it does the veratrine response (34).

Eccles and O'Connor (56) observed repetitive responses of a few of the fibers of cat muscles stimulated with single nerve volleys. They were able to localize the discharges to the muscles rather than the nerves. Two nerve volleys at a brief interval produced repetitive responses in a greater fraction of the fibers.

Tiegel's contracture has often been likened to the veratrine response (22). It consists of a slow relaxation after the twitch produced by a very strong but brief single electrical stimulus. It is generally considered to be a contracture due to the direct effect of the strong stimulating current on the contractile mechanism at the cathode. The similarities to the veratrine response are not so striking as in the case of Bremer's phenomenon (83). In view of the change in the status of the veratrine response, however, it seems desirable to reinvestigate with modern techniques the possibility that the response called Tiegel's contracture may be propagated.

In the clinical syndrome called myotonia the muscular response is strikingly like the veratrine response. Myotonia refers to the muscular responses occurring in persons with the familial diseases, Myotonia Congenita (Thomsen's disease) and Atrophia Myotonica (Curschmann's disease) (205). If these persons move slowly, their muscular contractions are normal. Sudden or strong muscular efforts, however, lead to prolonged contraction of the muscles involved, or, from the point of view of the patient, to the inability to relax the muscles for a period of many seconds. After repeated efforts without rest, relaxation gradually returns, and normal control of the muscles is possible. Thus with respect to summation and fatigue, myotonic responses are much like veratrine responses. The myotonic response is accompanied by a repetitive firing of the individual muscle fibers, even when it is set up by single volleys in the motor nerve. In the hereditary myotonia of goats, which is otherwise similar to the disease of man, the myotonic response occurs, with repetitive discharge of the muscle, even after complete curarization and in denervated muscles (35). That this muscular phase of myotonia does not explain the whole syndrome in man is apparent from the work of Denny-Brown and Nevin (49). Another locus of abnormally prolonged activity must be invoked; but it is not clear whether receptors, axons, or the central nervous system are the site of this abnormality.

The myotonic muscle of man and goat gives unusually prolonged and vigorous responses on mechanical stimulation. This, indeed, might be expected from muscles which respond in this manner to stimulation via the motor nerves. But it has not been clearly demonstrated that there is actually an increase of mechanical excitability, i.e., that less force is necessary to set off all-or-none responses in these muscles.

In myotonic muscles, as in veratrinized muscles, there is a relatively low anodal closing threshold, which may be lower than the cathodal closing threshold. That the mechanism of accommodation of muscle is affected in myotonia as in veratrinization is suggested by the slow contractions obtained with direct current (62).

Myotonic responses are like veratrine responses in being antagonized by calcium and by quinine (144, 205). In myotonic goats, the muscles are stimulated by intra-arterial doses of potassium chloride one-tenth as great as those which are necessary in the muscles of normal goats (35).

A second group of veratrinic responses may be produced by treating muscles with certain pharmacological agents. Glycerin and other agents which dehydrate frog muscle may produce a veratrinic response (228). Several aldehydes, including formaldehyde, and a number of inorganic ions, including perchlorate, have been shown to do the same (145, 252). These effects of glycerin and formaldehyde are antagonized by quinine (187). Certain derivatives of colchicine have been reported to have a veratrinic effect (80, 126).

Tetraethyl ammonium ion causes responses in frog and mammalian nerves which are indistinguishable from those of veratrinized nerves. Single, brief shocks elicit repetitive responses with an augmented negative afterpotential. Summation and fatigue of the veratrinic component are similar to those seen in veratrinization. Accommodation is significantly affected, and the anodal closing

threshold is decreased until it may be lower than the cathodal closing threshold. Calcium antagonizes the responses to tetraethyl ammonium (2, 41). Occasionally a veratrinic mechanogram may be obtained with this substance in the frog sartorius muscle (6). Tetraethyl ammonium resembles veratrum alkaloids also in causing a positive inotropic effect in the failing mammalian heart and, with higher doses, cardiac irregularities (4). On the blood pressure it has effects superficially similar to those of veratrum alkaloids but due to a different mechanism (5).

In 9-phenanthroate and some other 9-phenanthrene derivatives, Eddy (57) and Smith (239) found substances which in mammals cause a condition like human myotonia. No veratrinic mechanograms could be obtained from frog muscle; but the mechanical response of rabbit muscle, stimulated directly or indirectly, was like that found after veratrine. After curarization, the veratrinic response occurred on direct stimulation. Summation and fatigue occur in a manner similar to that described above for veratrine. Quinine opposes these veratrinic muscular effects. Unlike veratrine, 9-phenanthroate has no effect on the arterial pressure and heart rate of anesthetized cats and dogs (3).

Dihydronaphthacridine carbonic acid and a group of related substances (32) resemble closely the 9-phenanthroate in its veratrinic effects (113). It produces a myotonic syndrome in mammals which is associated with veratrinic responses of skeletal muscles that persist on direct stimulation after curarization or denervation. Summation and fatigue of the veratrinic component occur as with 9-phenanthroate or in clinical myotonia. Quinine opposes the veratrinic muscular effects (3). Anodal closing threshold is rendered lower than cathodal closing threshold, and a slowly accommodating response occurs on stimulation of muscles with direct current (175). In Europe this substance was used under the name "Tetrophan" in clinical neurology when a strengthening of muscular contraction was desired (see Foerster, 73).

Gasser (83) states that "the fact that such varied chemical entities come into a common group on the basis of their imitation of the action of veratrin indicates that they are revealing a fundamental characteristic of skeletal muscle." (See also 252). In the light of the discussion of section VI, A3, p. 412 we may tentatively revise this to apply also to nerve, or to the excitatory and conduction system of nerve and skeletal muscle. Further study may reveal dissimilarities among the veratrinic responses which will lead to a better understanding of this fundamental characteristic.[4]

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[4] As this goes to press we learn of the paper by Coppée (Arch. internat. de physiol. **53**: 327, 1943), parts of which deal with the action of veratrine on nerve and muscle. Welsh and Gordon (Fed. Proc. **5**: 112, 1946) have reported that DDT and pyrethrins, in common with veratrine, produce repetitive responses to single shocks in lobster nerve. Bucher (unpublished observations) has found that in mammals 2-4-dichlorphenoxyacetic acid produces veratrinic muscular responses.

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ARTHROPOD NERVOUS SYSTEMS: A REVIEW OF THEIR STRUCTURE AND FUNCTION[1]

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The immediate purpose of this review is to bring together such literature on arthropod nervous systems, and their physiology, as might facilitate studies on the mode of action of insecticides classed as neurotoxins. Of the several classes of arthropods far more is known concerning the neurophysiology of the Crustacea than of any other class. This is due largely to their more favorable size and their general distribution. Since such insecticides as the pyrethrins (Gaudin, 1937) and DDT (2,2 bis(p-chlorophenyl) 1,1,1 trichlorethane) (Welsh, unpublished) are more toxic to certain Crustacea than to insects, and since each substance appears to act in a similar manner in the two groups, it would seem quite justifiable to follow any suggestions which the studies on Crustacea offer in investigating the mode of action of these and other insecticides.

In the development of animal poisons it is desirable to have a material with a highly selective action on the group to be dealt with. There are some striking differences between the nervous systems of arthropods and vertebrates which should be kept in mind. Some of these are pointed out in the present review.

Aside from any practical use which might be made of this review it stands as the only recent attempt to bring together the literature in this field.

I. ANATOMICAL. 1. *Gross structure.* Typically the arthropod nervous system consists of *two* longitudinal cords ventral to the digestive tract with a dorsal brain. A pair of ganglia occur for each somite but where the somites are fused there is often a fusion of these ganglia, and where appendages are lacking the ganglia may be absent. Thus, in primitive Crustacea such as the "brine shrimp", *Artemia*, the nervous system has a ladder-like form; while in higher Crustacea and insects there is lateral fusion of the parallel cords and ganglia, and in crabs and certain higher insects much lengthwise shortening of the cord and fusion and loss of ganglia.

The brain is composed of the ganglia of the somites which are preoral; of paired ganglia for certain preoral, presegmental sense organs (eyes, frontal organs); and sometimes of a median anterior element, the *archicerebrum*. The principal parts of the brain are the *protocerebrum*, *deutocerebrum* and *tritocerebrum*. The identity of some of these ganglia may be lost in late stages of development.

2. *General arrangement of neurons.* Sensory neurons are numerous in arthropods and differ from vertebrates in having their cell bodies peripherally located. They are generally bipolar. The axons of sensory neurons enter the ventral ganglia through the *ventral* portion or "root" of a lateral nerve. Numer-

[1] This review was prepared as a part of a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the President and Fellows of Harvard College.

ous association neurons are found in the ganglia and most cell bodies belong to these, as the cell bodies of sensory neurons are peripheral and those of motor neurons few in number. Motor neurons are nearly all unipolar; their cell bodies are found in the peripheral portion of the ventral ganglia and the main stalk from the cell divides into a collateral and an axon filament. The *typically few* motor fibers leave a ganglion by the *dorsal* "root".

3. *Peculiar features of neuromotor system.* Striking differences from other animals are found in the arthropod neuro-muscular arrangements. In the first place an entire muscle may be innervated by branches from only *two* motor axons which may branch and rebranch to supply many endings on *each* muscle fiber. This was first shown by Biedermann (1887) in Crustacea and later extended by Mangold (1905) to a variety of arthropods. Biedermann suggested that of the two nerve fibers one was excitatory and the other inhibitory. This was confirmed by Hoffmann (1914) for Crustacea. Certain muscles of crustaceans may be innervated by three, four, or five motor fibers (van Harreveld, 1939c). When more than two motor fibers innervate a muscle all but one are excitatory and the stimulation of each separately produces a characteristic contraction and action current (e.g., van Harreveld and Wiersma, 1939).

Much less is known about the motor innervation of insects than is known about the same in Crustacea. Mangold (1905) using methylene blue staining described a double innervation of the thoracic and leg muscles of *Decticus* and of the wing muscles of *Dytiscus*. A recent study of the motor mechanism of the leg of *Periplaneta* by Pringle (1939) describes a double innervation of the extensor tibiae, one fiber producing a tonic contraction and the other a twitch. He found no evidence for the existence of inhibitory fibers but suggests that such may possibly exist in some insect muscle. It is obvious that further investigation of the histology and physiology of motor innervation of insect muscle is desirable.

4. *Nature of motor nerve endings.* By means of silver impregnation van Harreveld (1939a) studied the nerve endings on muscle fibers of the abductor of the dactylopodite of the crayfish. This muscle has a double innervation (one fiber excitatory, the other inhibitory). It was found that these fibers branched and rebranched simultaneously to end in a "tangle" of nerve fibers enveloping each muscle fiber. A single muscle fiber, 3.5 mm. long, was estimated to have at least forty nerve endings. This is a situation very different from that in the vertebrates where much longer fibers usually have one and, at the most, but two or three nerve endings. Evidence is presented by van Harreveld (1939a) indicating that at least part, and probably all, of the muscle fibers of a triply innervated muscle receive branches from the fibers for the fast and for the slow contraction. Further evidence for this, based on physiological experiments, will be presented later.

Mutiple nerve endings on a single insect muscle fiber have been described by a number of authors—most recently perhaps by Marcu (1929) who found in muscle fibers (1 mm. long) of the house fly as many as twenty nerve endings.

Van Harreveld (1939a) concludes that "the multiple innervation of the muscle fiber makes it anatomically possible that the impulse is not conducted

along the muscle fiber as is the case in vertebrate muscle, but that in the crayfish (and probably the insect) muscle the impulse is distributed by nerve conduction to a great number of points on the muscle fiber. The action current of the crayfish muscle would then not be the sign of a conducted process in the muscle fiber, but of a local excitatory process."

Wigglesworth (1939) says, regarding nerve endings in insect muscle, "when the nerves reach the ordinary muscles they break up into smaller branches which penetrate between the fibers and have their final terminations anywhere along them. The nerve often ends in a conical projection, the 'end plate' or Doyère's cone; the nerve fibre penetrates the sarcolemma and breaks up into fine branches with varicose thickenings of all sizes and shapes. These 'end plates' often contain nuclei belonging to the nerve sheath or neurilemma, and strands of cytoplasm may radiate from them into the substance of the muscle fibre. In the fibrillar indirect muscles of the wings the nerve endings are difficult to find, probably they take the form of delicate branches which end all over the fibre surface. Filiform endings of this kind may occur also in any of the types of muscle."

5. *Innervation of the heart.* Brief mention may be made concerning the innervation of the arthropod heart since this organ is frequently studied in connection with drug and ion effects.

Among the Crustacea the situation in the Decapoda is best known. All marine decapods, thus far investigated, have an intrinsic cardiac ganglion composed of nine cells (five large and four small) (Alexandrowicz, 1932; Welsh, 1939). The crayfish has as many as sixteen cells composing its cardiac ganglion (Alexandrowicz, 1929). Extrinsic excitatory and inhibitory nerves from ventral ganglia (CNS) end on, and regulate the activity of, the intrinsic neurons (Alexandrowicz, 1932; Wiersma and Novitski, 1942; Smith, unpublished thesis)[2].

Among the few lower Crustacea which have been studied, only in Daphnia are ganglionic pacemaker cells apparently lacking in the heart (Prosser, 1942; Baylor, 1942).

Since the early classical studies of Carlson the *Limulus* heart has been accepted as a typical neurogenic heart with intrinsic ganglion and extrinsic regulatory nerves. This is the only arachnoid concerning which information on motor regulation of the heart is at hand.

Concerning the presence of intrinsic ganglionic cells in insect hearts one may refer to Wigglesworth (1939) or to Yeager and Gahan (1937) for all that seems to be known. Alexandrowicz (1926) reports the presence of ganglionic heart cells in a cockroach but most other workers have failed to obtain convincing histological evidence of the presence of such cells in other species. Yeager and Gahan (1937) suggest that the different effects of nicotine on isolated hearts of *Periplaneta* and larval *Prodenia* may be due to the presence of ganglionic cells in the former and their absence in the latter. In any study involving the cardiac mechanism of insects the question of the occurrence of pacemaker neurons should be given consideration.

[2] Smith, R. I. 1942 Studies on the cardio-regulatory nerves of decapod crustaceans. Thesis. Harvard University.

II. The Nerve Cell and its Sheath. Although the arthropod neuron was formerly believed to differ considerably from that of the vertebrates, recent work has indicated that the difference is one of degree rather than of kind. The principal investigations have been those of Schmitt and Bear, in which the polarizing microscope was used to show that the crustacean nerve, like that of the vertebrates, is surrounded by a lipo-protein sheath. These and other studies are reviewed in the following section. Cytoplasmic structures in neurons of Orthoptera are discussed by Beams and King (1932), while those of Crustacea are treated by Lacroix (1935). These papers contain references to earlier work on this subject. Among the characteristics distinguishing the arthropod neuron from the vertebrate are the following:

1. Instead of converging to form an axon hillock near the periphery of the cell, the neurofibrillae form an intracellular axon deep within the cell body.
2. The Golgi apparatus does not form a reticulum, but is in discrete particles.
3. A supporting network, known as the trophospongium, stretches inward from the border of the cell.

Although the invertebrates were generally considered to have only unmyelinated nerves, Retzius (1888) found myelinated fibers in the crustacean *Palaemon squilla*. Further work showed that as in the vertebrates, the myelin is broken by nodes between the sheath cells. Unlike the vertebrates, the nucleated sheath cells are not outside the myelin, but lie between it and the axon (Retzius, 1890). In the leg nerves of *Maia*, on the other hand, Young (1936) found that the axons "are enclosed in continuous sheaths, formed of sheets of a collagen-like substance, interspersed with nuclei, containing some fat, and uninterrupted by any breaks comparable with the nodes of Ranvier."

With the aid of the polarizing microscope, Schmitt and Bear (1937) found that even the so-called "unmyelinated" fibers of vertebrates are myelinated. In small fibers of the frog sciatic, the nerve sheath is mostly protein. In fibers of progressively greater diamater, the amount of lipid in the sheath becomes progressively greater. Hence there is a continuous gradation between the small "unmyelinated" and the large myelinated fibers. The lipid micelles are oriented radially, the protein tangentially, to the cell axis.

Bear and Schmitt (1937) showed that the thin sheath of crustacean axons has the same properties as the myelin sheath of vertebrates. By immersing the nerve in glycerine, which has the same refractive index as protein, the protein birefringence was reduced, revealing the lipid orientation. Similarly, by treatment with lipid solvents such as alcohol or ether, the lipids were removed revealing the protein orientation. These methods showed that the optical properties of the sheath are caused by:

1, a form birefringence resulting from the shape and orientation of the protein micelles;

2, an intrinsic birefringence of the lipid micelles.

Further studies indicated that in addition to their form birefrigence, there is an intrinsic birefrigence of the protein micelles (Chinn and Schmitt, 1937). The nerve cell bodies of crustaceans are enclosed in a thin sheath continuous with

and similar in its optical properties to the axon sheath. Oriented protein is also found in the cytoplasm and nuclear membrane (Chinn, 1938).

Taylor (1941) found that as in the vertebrates, the lipids of shrimp nerve sheaths are oriented radially, the proteins tangentially. Likewise, as in the vertebrates, the degree of myelination increases with the size of the fiber. However, a shrimp nerve sheath has much less myelin than that of a frog or cat nerve of the same diameter. Small fibers of both crustaceans and vertebrates have a relatively thicker sheath than do large fibers, although a vertebrate fiber of the same diameter as a crustacean fiber has a thicker sheath. The range of values for the ventral cord fibers of the shrimp is as follows:

FIBER DIAMETER	AXON DIAMETER	$\frac{\text{AXON DIAMETER}}{\text{FIBER DIAMETER}}$	BIREFRINGENCE
μ	μ		
8.3	5.5	0.66	−0.00002
60.0	51.1	0.85	+0.0019

A different situation exists in the leg nerves of *Maia*, where Young (1936) found that large fibers have relatively thicker sheaths than small ones.

Richards (1943) showed that insect nerve cells are surrounded by a lipid sheath, probably composed of phospholipids and cholesterol. Using xylol marked with a sudan stain, he showed that oil-soluble insecticides, entering through the tracheae, penetrate the nerve cell through this sheath. Studies on the birefringence of the insect nerve sheath (Richards, 1944) showed that its properties are similar to those of crustacean sheaths. The principle differences are that both protein and lipid micelles are oriented radially, and that the birefringence of the protein in insects is less than that in crustaceans. Furthermore, the insect nerve cord is surrounded by a permeable, elastic neural lamella, composed of a protein differing from that of the nerve sheath. Comparative studies indicated that lipo-protein nerve sheaths are found in all the major groups of the annelid-arthropod complex.

III. Biochemistry. Perhaps the most striking indication in the biochemistry of crustacean nerve is that its metabolism is less efficient than that of vertebrate nerve. The carbohydrate reserves of crustacean nerve are much greater, as is its respiration and heat production. The creatine of vertebrate nerve is replaced by arginine in crustacean nerve. Hence crustacean nerve is much more fatigable than vertebrate nerve.

Other differences are that crustacean nerve contains large amounts of unidentified anions of unknown function. Acetylcholine and cholinesterase may be much more concentrated in arthropod central nervous systems than in those of vertebrates. The following section considers these and other facts in greater detail.

1. *Ionic composition.* When nerves are soaked in isotonic sugar solution, the Cl and most of the Na readily escapes, while the K is retained (Fenn, Cobb, Hegnauer and Marsh, 1934). This and other evidence indicates

that Na and Cl are concentrated outside the fibers and K inside. In frog nerve the "K space" is 36 per cent of the weight of the nerve, while in crab nerve it is 75 per cent. This is probably correlated with the large diameter and thin sheath of the crab axons.

Some analyses made by the above authors follow. The values for crab blood are from Cole (1940), while those for insect blood are from data quoted by Heilbrunn (1943):

	Na	K	Ca	Mg
	mM./kgm.	*mM./kgm.*	*mM./kgm.*	*mM./kgm.*
Crab blood	460	12	12	22
Crab nerve	168	158	67.5	—
Nerve/blood ratio	0.35	13	5.6	—
Frog plasma	103.8	2.5	2.0	3.0
Frog nerve	62.0	48.0	3.6	8.0
Nerve/blood ratio	0.60	19	1.8	2.7
Insect blood	22.3	39.7	9.2	7.1

In keeping with the ion content of their bloods, the total ion content of crab nerve is greater than that of frog nerve. Na and K are less concentrated relative to the blood in crab nerve than in frog nerve, while Ca is more concentrated. The high concentration of K in the nerves of these animals is particularly noteworthy. In view of the high concentration of K relative to other ions in insect blood, studies of the ionic composition of insect nerve would be of great interest.

Analysis of the balance between anions and cations in lobster nerve (Schmitt, Bear and Silber, 1939) gives the following results:

	LOBSTER NERVE	SEA WATER
	mEq./g.	*mEq./g.*
Total base	0.56	0.58
Cl	0.17	0.52
Anion deficit	0.39	0.03

Further studies indicate that this anion deficit is composed of free amino acids. The concentration of these acids in lobster nerve is 60× that in the blood (Silber and Schmitt, 1940). It is suggested that these anions may play a rôle in maintaining bioelectric potentials. Free amino acids are not found in medullated nerve, where their place may be taken by phospholipids. Silber (1941) found that while ⅓ of this anion deficit could be accounted for by amino acids, particularly alanine and aspartic acid, the other ⅔ was composed of as yet unknown compounds. In connection with the above work, it is interesting to note that choline acetylase, the enzyme which synthesizes acetylcholine, is stimulated by glutamic and other amino acids (Nachmansohn and John, 1945).

2. *Carbohydrates.* Crab leg nerve is much richer in carbohydrate than is vertebrate medullated nerve. The following data are from Holmes (1929):

	CANCER LEG NERVE	VERTEBRATE MEDULLATED NERVE
	mgm./100 g.	*mgm./100 g.*
Glycogen	299–2192	59
Free carbohydrate	206–239	42

Glycogen may comprise 20 per cent of the total solid in crab nerve. In mosquito larvae, Wigglesworth (1942) found that glycogen is stored in the ganglia and axons of the central nervous system, occurring in the form of granules and small vacuoles. This material is used up in starvation, but is reformed on feeding starch or alanine.

3. *Lipids.* The lipids of the honey bee brain are remarkably similar to those of the brain of a young vertebrate prior to the appearance of visible medullation. The chief difference is that the sterols are lower (Patterson, Drumm and Richards, 1945).

4. *Proteins.* Lobster leg nerve contains 12.6 mgm./g. total N (Schmitt, Bear and Silber, 1939). This compares with 13.0 mgm./g. in the spinal cord and 20.6 mgm./g. in the peripheral nerve of cattle (Abderhalden and Weil, 1912).

A protein complex with the properties of a nucleoprotein was extracted from lobster leg nerve by Bear, Schmitt and Young (1937). It is unstable in acid, precipitating as a metaprotein. A histone or protamine-like group is split off in alkali. A fraction containing P and characteristic of a nucleic acid can be split off the bulk of the protein. The same complex was extracted from squid giant fiber axoplasm and mammalian central nervous system. Since it is apparently characteristic of neuroplasm generally, it was called *neuronin*.

5. *Acetylcholine (Ach) and Cholinesterase (ChE).* Marnay and Nachmansohn (1937) found that the ChE content of lobster ventral nerve cord is 20× that of frog sciatic. This is the specific ChE which splits Ach more rapidly than other esters, and not a less specific esterase which splits other esters more rapidly than Ach (Nachmansohn and Rothenburg, 1945).

Ach was extracted from crab nervous tissue by Welsh (1939a) and from crayfish and lobster nervous tissue by Smith (1939). Smith obtained the following values:

	FIBERS	GANGLIA
	γ/g.	γ/g.
Cambarus fall	4	14
Cambarus spring	13	46
Homarus spring	9	17

Schallek (1945) found that the ventral ganglia of the crab *Cancer irroratus* averaged 3.1 γ/g. while the ventral nerve cord of the lobster *Homarus americanus* averaged 15.9 γ/g. Corteggiani and Serfaty (1939) found Ach and ChE in the nervous systems of various insects, obtaining as much as 200 γ/g. Ach in *Carausius.* Comparable amounts were found in the ventral nerve cord of *Periplaneta* by Mikalonis and Brown (1941).

These values stand in great contrast to those in the vertebrate central nervous system. MacIntosh (1941) found that the Ach level of the cat brain varies from 0.18 γ/g. in the cerebellum to 7.0 γ/g. in the basal ganglia. Higher values are found in the peripheral nervous system, reaching 40 γ/g. in automomic ganglia. Welsh and Hyde (1944b) obtained up to 800 γ/g. in the myenteric plexus of the guinea pig.

Ach occurs in both the free form and in an inactive form in which it is bound to protein. Although assays of arthropod nerve by Corteggiani (1938) and Corteggiani and Serfaty (1939) indicate that all the Ach in these nerves is in the free form, Schallek (1945) found that in the ventral nerve cord of *Limulus* about 40 per cent of the Ach was in the free form; of *Cambarus*, about 50 per cent; and of *Homarus*, about 60 per cent. In the brain of the adult rat from $\frac{1}{3}$ to $\frac{1}{2}$ of the Ach is in the free form (Welsh and Hyde, 1944a), while in the sciatic nerve about $\frac{2}{3}$ is in the free form (Prajmovsky, unpublished).

ChE in *Melanoplus* is 8× more concentrated in the nervous system than in muscle. The highest concentrations in the nervous system are found in the brain (Means, 1942). ChE appears in the *Melanoplus* embryo in small amounts before the nervous system is differentiated, and then increases rapidly as the nervous system develops. Ach does not appear until later (Tahmisian, 1943).

6. *Adrenalin.* Very little is known about the occurrence of adrenalin in arthropods. 3, 4 dihydroxyphenylalanine, a possible precursor of adrenalin, was obtained from the larvae of the insect *Tenebrio* by Raper (1926), while a substance having the pharmacological and chemical properties of adrenalin was extracted from the same species by Wense (1938). However, as the extracts were made from whole larvae, it is not certain that these substances came from nervous tissue.

7. *Metabolism.* The respiration of crab nerve is 10× that of frog nerve at the same temperature (Meyerhof and Schulz, 1929). Following a 15 minute stimulation, the increased O_2 consumption is 20× that of frog nerve. This increased respiration rises to a maximum 15 minutes after the end of stimulation, and may last for 2 hours. Similar relations were found in measurements of the heat production of crab nerve (Beresina and Feng, 1933). The resting heat is 3× that of frog nerve. The total heat liberated by a short stimulus is over 30× that of frog nerve. While recovery heat in the frog starts at a maximum, that in the crab starts low and gradually rises to a peak. Crab nerve is much more susceptible to fatigue than frog nerve. This seems to be correlated with the slow recovery heat of crab nerve. At low temperatures, where the recovery heat is even more prolonged, fatigability is more pronounced.

Bronk (1932) showed that during a series of contractions in crab muscle, the heat production decreases, due to a slowing of the muscle. This increasing economy of contraction is much greater than in vertebrate striated muscle; it enables the animal to maintain a sustained contraction despite the rapid fatigue of its nerve. Furthermore, the crustacean muscle can produce either a rapid twitch or a slow contraction. Keighly and Wiersma (1941) found that for similar work the slow contraction is half as expensive as the fast.

Monaghan and Schmitt (1931) showed that while *Limulus* and *Homarus* muscle contains cytochrome, their leg nerve contains a hemochromagen which is not cytochrome. Frog sciatic nerve has no hemochromagen, but contains a carotinoid which may be carotin. This pigment seems to be involved in oxidative processes.

The buffering properties of crab nerve and muscle were studied by Cowan (1933). The CO_2 combining capacity of crab nerve is nearly the same as summer frog nerve, while that of crab muscle is 3 per cent greater than frog muscle.

Engel and Gerard (1935) found that the creatine of vertebrate nerve is replaced by arginine in crustacean nerve. This implies that the crustaceans have a less efficient metabolism than the vertebrates. On stimulation, phosphoarginine is decomposed, while during recovery in O_2 it is resynthesized at the expense of adenosinetriphosphate (ATP). Further studies on lobster muscle (Ochoa, 1938) revealed the following differences between its metabolism and that of vertebrate muscle:

In vertebrates:

$$\text{2 phosphopyruvate} + \text{adenylic acid} \rightarrow \text{2 pyruvate} + \text{ATP}$$
$$\text{ATP} + \text{2 creatine} \rightleftharpoons \text{adenylic acid} + \text{2 phosphocreatine}$$

In lobster:

$$\text{phosphopyruvate} + \text{ADP} \rightarrow \text{pyruvate} + \text{ATP}$$
$$\text{ATP} + \text{arginine} \rightleftharpoons \text{ADP} + \text{phosphoarginine}$$

IV. Electrical Phenomena. Although a considerable amount of investigation has been done on the electrical properties of arthropod nerve, general conclusions are hard to draw. An outstanding feature of crustacean nerve is that it usually responds to a single stimulus with a series of impulses. This may be due to its accommodation time being much slower than that of vertebrate nerve. The conduction velocity of arthropod fibers is less than that of vertebrate fibers of the same diameter. This is correlated with a much slower accommodation time than that of vetebrate nerve.

Electrical studies of sense perception in arthropods indicate that there are primitive sound receptors which cannot distinguish between different frequencies, as well as more elaborate receptors permitting true hearing. Similarly, there are primitive photoreceptors as well as true eyes.

The central nervous system of arthropods is notable for the persistent rhythmic electrical discharge of isolated nerve cords. Apparently certain cells in the cord discharge without sensory stimulation. This spontaneous activity is greatly influenced by changes in the ionic composition of the surrounding fluid.

A. *Peripheral Nerve.* 1. *Nerve potentials.* Cowan (1934) found that the injury potential of *Maia* (European spider crab) nerve is about 30 mV. Bogue and Rosenberg (1936b) showed that the action potential in mixed *Maia* leg nerve is only about 10 per cent of this injury potential. In thin sensory bundles the action potential is higher, ranging from 10 to 15 mV.

The injury potential of crustacean nerve falls during stimulation or during

O_2 lack; recovery occurs only in the presence of O_2 (Furusawa, 1929). Hence the maintenance of the membrane potential depends upon oxidative processes.

The K concentration inside *Maia* nerve is 13× that in the blood (Cowan, 1934). During stimulation, K leaks from the nerve. Increasing the external K lowers both the injury and action potentials. The resting potential also drops in the absence of O_2 but K does not leak from the nerve. The depression in asphyxia is therefore not due to the loss of K.

Spider crab nerve is blocked at low temperature (Ets, 1936). Increasing the external K causes this block to occur at a higher temperature. Crab nerve is more sensitive to K than frog nerve, since with a perfusion fluid containing 10× the normal K, crab nerve is blocked in 10 min., while frog nerve is blocked in 108–180 min. This difference may be due to the thicker myelin sheath of frog nerve.

The action of K in lowering the resting potential is opposed by Ca (Cowan, 1934). Ca by itself has no effect on the resting potential, but it does cause a reversible depression of excitability (Guttman, 1940). Mg increases the electrical response of crab nerve (Katz, 1936a).

Passage of the action current in crab nerve is followed by a slowly declining negativity (Levin, 1927). If a second impulse follows the first before this "retention" has passed away, the size of the second impulse is correspondingly reduced. Levin considers that this negative after-potential is the electrical sign of fatigue. Although present in frog nerve, it is much less marked because of the greater resistance to fatigue of this nerve. During the passage of a series of impulses, the negative potential gradully declines and is replaced by a positive after-potential (Arvanitaki, 1936). In crab nerve which has been poisoned by yohimbine, a positive potential appears after a single nerve impulse (Bayliss, Cowan and Scott, 1935).

2. *Excitation.* Sub-threshold stimuli set up non-propagated potential changes in crustacean nerve (Hodgkin, 1938). A weak stimulus produces a polarization potential which behaves as though it were due to passive accumulation of charge at the nerve membrane. A stronger stimulus produces an additional potential behaving like a subliminal response of the nerve fiber. These two potentials have different properties. Thus the polarization potential falls at the end of the shock, while the subliminal response continues to rise. The former is a passive result of the applied current, the latter an active response of the nerve.

Bogue and Rosenberg (1936a) find that in thin sensory fibers of *Maia* nerve, the interval between the make of a constant current and the response is 23–74 msec. at rheobasic strength. The corresponding time in frog sciatic nerve is 1.7–2.0 msec.

The following measurements of the chronaxie of arthropod leg nerves were made by Monnier and Dubuisson (1931):

	FAST WAVE	SLOW WAVE
	msec.	msec.
Limulus	1.20	3.75
Callinectes	0.70	2.15
Homarus	0.35	1.70

Chronaxies of nerve in the dog, for comparison, are 2 msec. for sciatic vasomotor fibers and 0.15 msec. for the facial nerve (data from Brücke, 1930).

Cutting a motor nerve of *Cancer* gives rise to a series of impulses, lasting from 15 sec. to 20 min. (Barnes, 1930b). Similarly, the nerve of *Callinectes* responds to a single stimulus with a series of impulses (Jasper and Monnier, 1933). Because of this, the excitation time of crustacean nerve is a "pseudo-chronaxie" when determined by muscular contraction. For *Callinectes* it equals 0.6–0.8 msec.

Arvanitaki (1936) found that in *Carcinus* and *Maia*, this multiple response occurs only after the third or fourth of a series of stimuli. Preceding and following this chain of spike potentials is a series of smaller potentials with the same periodicity as the spikes. When the spike potential appears, it seems to be superimposed on these smaller oscillations.

Solandt (1936) finds that this multiple response of crustacean nerve is correlated with a much slower accommodation time than that of vertebrate nerve. Values for the accommodation time (λ) of several nerves follow:

	msec.
Winter frog sciatic	35
Maia squinado	1665
Cancer pagurus	3225
Homarus vulgaris	3710
Carcinus maenas	4160

Raising the Ca concentration of the perfusate decreases the accommodation time, although the effect is much less in crab nerve than in frog nerve. Application of 15×, the normal Ca to crab nerve abolished the multiple response, the nerve giving single impulses only (Katz, 1936a). Multiple response was obtained in frog nerve by either chilling the frog or placing the nerve in low Ca. Multiple response could then be abolished by raising the temperature or increasing the Ca concentration (Katz, 1936b).

3. *Conduction velocity.* Some data on conduction velocity in arthropod leg nerves follow. Stimulation of a nerve may give rise to several waves, due to different fiber groups conducting impulses at different velocities:

	1ST	2ND	3RD WAVE	AUTHORITY
	meters/sec.	meters/sec.	meters/sec.	
Limulus	4.60	1.30		Monnier and Dubuisson, 1931
Callinectes	5.00	1.50		Monnier and Dubuisson, 1931
Homarus	9.20	1.85		Monnier and Dubuisson, 1931
Homarus	6.40			Auger and Fessard, 1934
Maia	4.20			Auger and Fessard, 1934
Maia	3.7–2.5	1.7–1.1	0.5–0.1	Lullies, 1933
Maia	2.5			Bayliss, Cowan and Scott, 1935
Maia	5.29	2.09	1.39	Bogue and Rosenberg, 1936b

The nerves of *Leander serratus*, which are more heavily myelinated than the above nerves, have a conduction velocity of 18–23 meters/sec. (Holmes, Pumphrey and Young, 1941). Mammalian A fibers, in contrast, can conduct as fast as 100 meters/sec. (Grundfest, 1940).

Barnes (1932b) showed that the rapid closing reflex of the crustacean claw involves sensory fibers of larger diameter than those used in the slower opening reflex. Conduction velocity, however, depends not only upon fiber diameter but also upon the degree of myelination (Taylor, 1942). The following table compares various fibers chosen to have the same conduction velocity. Myelination is measured by positive birefringence.

FIBER	FIBER DIAMETER d_1	AXON DIAMETER d_2	$\frac{d_2}{d_1}$	BIREFRINGENCE Na-No	CONDUCTION VELOCITY	(Na-No)d_1
	μ	μ			*met./sec.*	
Squid giant	650	637	0.98	−.0001	25	
Earthworm giant	100	90	0.90	+.0010	25	0.10
Shrimp giant	50	43	0.87	+.0024	25	0.12
Frog sciatic	10	7.5	0.75	+.0105	25	0.105
Cat saphenous	8.7	6.6	0.76	+.0140	25	0.12

It is evident that a small fiber can conduct as fast as a large one if it is more heavily myelinated.

Monnier and Dubuisson (1931) find that animals can be classified according to the distance traversed by an impulse during the chronaxie of the nerve:

	mm.
Lobster, both waves	3.5
Crab, both waves	3.5
Limulus, both waves	5.0
Shark, α wave, lateral line nerve	8.0
Frog, α wave, sciatic nerve	12.0
Dog, α wave, femoral nerve	16.0

The increase in speed of conduction in the course of evolution is evident.

B. *Sense Perception.* 1. *Touch and proprioception.* Adrian (1930) recorded sensory discharges in the central nervous system of the caterpillar on touching the tail or head. Proprioceptive impulses are set up in the nerves of various crustaceans on bending the limb (Barnes, 1930a, 1932a). Unlike vertebrate tension receptors, these end organs are in the skin of the joint, and show rapid adaptation. Sensory impulses set up by these organs on moving the isolated limb cause a reflex motor response in the absence of the central nervous system. Possibly the motor nerve is stimulated by action potentials in the many fine sensory nerves which surround it.

Prosser (1935a, c) recorded proprioceptive impulses in the crayfish following flexion of an appendage. Tactile stimuli were recorded following movement of sensory hairs. The excitation time of caudal sensory hairs is 0.5–1.5 msec.

Pringle (1938a, b, c) found that the fifth segment of the palp of *Periplaneta* is covered by hairs sensitive to touch. Movement of these hairs gives rise to nerve impulses of small amplitude; their adaptation is rapid and complete. A group of campaniform sensilla on each joint of the palp respond to bending of the joint or pressure on the cuticle with large, rhythmic impulses of constant height; their adaptation is slow and incomplete. Other sensilla are found on the legs.

2. *Hearing.* The electrical response of insect nerves to stimulation of auditory receptors was studied by Pumphrey and Rawdon-Smith (1936). The tympanal organ of the locust responds to tones over a frequency range of 500–11,000 cycles/sec. Since the response is asynchronous at all frequencies, the organ is unable to distinguish between different frequencies. The anal cercus of the cricket and cockroach, on the other hand, responds synchronously over a wide frequency range. No lower limit of frequency could be found, and there is little response above 3000 cycles/sec. In the lower range of frequency the response is almost completely synchronized with the stimulus. Occasionally, however, short bursts of nerve activity will occur at twice the stimulus frequency, while the response to high frequencies may have only one-half or one-quarter the frequency of the stimulus.

3. *Photoreceptors.* Prosser (1934b) showed that the sixth abdominal ganglion of the crayfish acts as a photoreceptor. Illumination of the ganglion causes a burst of impulses which reach a maximum in about 2 sec.; adaptation is slow and incomplete. The activity continues for several seconds after the light is turned off. The response of the eye, as recorded in the optic nerve, is quite different. A rapid burst of impulses starts 0.03 sec. after turning on the light. The frequency reaches a peak within 0.1 sec., and then falls off rapidly, reaching a new level in about 0.5 sec. A similar but smaller burst occurs when the light is turned off.

Although visual physiology is outside the scope of this review, the following electrical studies in arthropods may be mentioned: *Limulus* (Hartline and Graham, 1932); *Dytiscus* (Adrian, 1937); *Melanoplus* (Roeder, 1939); various insects (Crescitelli and Jahn, 1942).

C. *Central Nervous System.* 1. *Excitation and conduction.* Although injury to peripheral nerve in the crayfish is followed by a high frequency discharge which may last several minutes, cutting a central commissure produces an asynchronous discharge lasting only a few seconds (Prosser, 1934a). Furthermore, this injury response can be obtained in an isolated claw nerve, but not in an isolated central commissure.

Conduction velocity in the ventral nerve cord of the crayfish is 3–10 meters/sec. (Prosser, 1935c). This is somewhat higher than the velocities found in arthropod peripheral nerve. The time per segment is 4.0–5.5 msec. of which ganglionic delay is 3.5–4.5 msec. A few fibers, however, pass through each ganglion except the last without synapse.

Afferent impulses set up by stimulation of caudal sensory hairs were recorded as they entered the caudal ganglion. The response adapts at a steady rate during repetitive stimulation and fails to synchronize with the stimulus at frequencies above 100/sec. The non-functional recovery period for most of the units is less than 5 msec., while the relative recovery period lasts for another 5 msec. Anterior to the ganglion, the afferent response fails to synchronize with repetitive stimuli faster than 10/sec. The non-functional recovery period is approximately 0.1 sec., while no relative recovery period could be observed. Prosser suggests that these differences are due to fiber attenuation.

Pumphrey and Rawdon-Smith (1937) studied the transmission of nerve im-

pulses through the last abdominal ganglion of the cockroach. The post-ganglionic response is double, consisting of a small wave due to a few "through" fibers, followed by a larger wave due to fibers synapsing at the ganglion. The "excitatory potential" built up at the synapse by afferent impulses reaches a maximum within 3–4 msec., since synaptic delay is never longer. The effects of the potential persist at least 20 msec., as this is the maximal interval between stimuli at which summation will occur. At low stimulus frequency the post-ganglionic response declines, although the pre-ganglionic response is unaltered. Increasing the frequency or intensity of stimulation will now restore the post-ganglionic response. Apparently the post-ganglionic neurons become adapted to the original stimuli, and the "excitatory potential" must now reach a higher level to maintain the initial response.

2. *Spontaneous activity.* Rhythmic discharges in isolated arthropod ventral nerve cords have been noted by several authors:

Caterpillar	Adrian, 1930
Dytiscus	Adrian, 1931
Cambarus	Prosser, 1934a
Periplaneta	Roeder and Roeder 1939
Limulus (cardiac ganglion)	Heinbecker, 1936

Roeder and Roeder (1939) noted an arrhythmic background of action currents of 30–60 mV in the isolated ventral nerve cord of the cockroach. Occasional rhythmic bursts of spikes would appear, with a frequency of 10–20/sec. and an amplitude of 100–150 mV. Other bursts of activity were apparently associated with the respiratory movements of the intact animal.

A detailed study of spontaneous activity in the crayfish ventral nerve cord has been made by Prosser. In the first of these studies (1934a) he found that there is a continual discharge of spikes of about 10 mV in amplitude and less than 1 msec. in duration. Analysis of the records showed that the discharge is due to individual ganglion cells firing off at a frequency of 10–20/sec. Although the discharge from each cell persists only a few seconds, shifting from one cell to another keeps the total frequency constant. Apparently certain cells in the central nervous system can discharge without sensory stimulation.

Prosser (1935b) showed that increasing the temperature increases the number of impulses in deafferented crayfish ganglia. Prosser and Buehl (1939) found that this spontaneous activity is dependent on O_2, as it declines rapidly in N_2. The activity is depressed by cyanide and carbon monoxide, indicating that respiratory enzymes are involved.

The effect of ions on this spontaneous discharge in the crayfish has been investigated by Roeder (1941) and Prosser (1943b). High K or low Ca initially stimulate the activity but later depress it. Low K or high Ca stimulate fresh preparations but depress older ones. Ca antagonizes the depressant action of high K but not its excitant action; neither action of low K is completely antagonized by low Ca. Among the anions, thiocyanate has a marked stimulating effect. Evidently the actions of these ions are quite complex.

In the heart ganglion of *Limulus*, the discharge of neurons is increased by high

K or low Ca, and decreased by low K or high Ca (Prosser, 1943a). These relations seem to be somewhat simpler than in the crayfish nerve cord.

V. NEURO-MUSCULAR SYSTEMS. A. *Crustacea.* Some essential differences between crustacean and vertebrate nerve-muscle systems are listed by Ellis, Thienes and Wiersma (1942):

1. Crustacean muscles are innervated by two to five axons, each of which innervates every fiber in the muscle. Vertebrate striated muscle is innervated by a large number of axons, each of which innervates only a portion of the fibers in the muscle.

2. Each crustacean muscle fiber receives many branches from each of the axons innervating it; each vertebrate muscle fiber is usually innervated by a single motor ending.

3. While vertebrate skeletal muscle fibers contract in only one way, crustacean skeletal muscle fibers can produce either a rapid twitch or a slow contraction.

4. Vertebrate striated muscle responds to a single nerve impulse with a maximal twitch. Crustacean muscle usually requires facilitation; that is, several nerve impulses are needed before contraction occurs.

5. While inhibition in vertebrates is primarily a central phenomenon, that in crustaceans is peripheral. Stimulation of a specific inhibitory neuron inhibits the response of the muscle to the other neurons.

These differences are considered in greater detail in the following sections.

1. *Multiple innervation.* Lucas (1917) showed that *Astacus* muscle can respond to stimulation with two distinct types of contraction. Pantin (1936b) found that the fast and slow contractions of the closer muscle of the *Carcinus* claw show marked differences in their rates of contraction and in their response to intensity and frequency of stimulation.

Van Harreveld and Wiersma (1936) showed that these two types of contraction are obtained by stimulation of different axons innervating the muscle. The same authors (1937) found that three of the muscles of the *Cambarus* claw show a triple innervation. Stimulation of the thickest axon produces a fast contraction, of the next in size a slow contraction, and of the smallest, inhibition. Other muscles in the claw have only a double innervation. A different arrangement is found in the claw of crabs (Wiersma and Marmont, 1938), while the flexor of *Panulirus* shows a quintuple innervation (van Harreveld, 1939c; van Harreveld and Wiersma, 1939). Four of the fibers are excitatory, each producing a different type of response, while the fifth is inhibitory. Because of these differences in innervation, conclusions drawn from experiments on one arthropod nerve-muscle system are not necessarily applicable to others.

In vertebrate skeletal muscle, which is innervated by a large number of axons, different types of response are obtained by variation in the number of active axons or in the frequency of their discharge. In crustacean muscle, which is innervated by only a few axons, a different type of response is elicited by each axon.

Knowlton (1942) points out that the slow contraction is the more primitive type, being associated with smooth muscle. The rapid twitch develops with

striation; the primitive arthropod striated muscle may show both types of contraction.

2. *Facilitation.* Pantin (1934) found that the latent period of *Carcinus* leg muscle to repetitive stimulation applied directly to the muscle is 7–10 msec., comparable to the value for frog muscle. When stimulated through the nerve, the latent period rises to 50–300 msec. Allowing 4–5 msec. for nerve conduction, it is evident that the bulk of this delay is at the neuro-myal junction. Evidently many stimuli must reach the muscle before a contraction occurs.

Further work (Pantin, 1936a) showed that a single nerve impulse produces little or no response in the muscle. A succession of shocks causes a contraction, the rate and tension of which increase progressively with the frequency of stimulation. Evidently each successive impulse brings more and more muscle fibers into action.

In vertebrates, each skeletal muscle is composed of a multiplicity of motor units. As the intensity of the stimulus increases, more and more motor units are activated, and the tension developed thereby increases. Increasing the frequency of maximal stimuli develops a tetanus through fusion of successive contractions. Because of the small numbers of axons in crustacean muscle, this mechanism does not hold. The entire muscle acts as a single motor unit. As long as stimulation is through one axon, the tension developed is independent of the intensity of the stimulus. The response is governed entirely by the frequency and duration of stimulation. Gradation is brought about through neuro-muscular facilitation.

A single action potential in crustacean nerve produces a single action potential in muscle (Du Buy, 1935). The muscle action potential consists of a small spike, presumably due to the nerve impulse, followed by a longer potential which may be produced by a chemical mediator. This latter potential seems to be an index of the facilitation process.

Blaschko, Cattell and Kahn (1931) showed that the interjection of a single stimulus during low-frequency stimulation of crab nerve produces a rapid increase in muscle tension which is subsequently maintained by the low-frequency stimulus. Pantin (1936b) suggests that this effect is due to facilitation. Most of the muscle fibers are not excited by the initial low-frequency stimulus, but they are so facilitated that the single interjected impulse is enough to excite them. Once the conduction path has been established, the low-frequency excitation is sufficient to maintain it.

3. *Excitation.* Wiersma and van Harreveld (1938a, b) showed that a single impulse in the thick (fast contraction) axon of the *Cancer* leg sets up an action current in the muscle, but does not cause a contraction. If a second shock follows within 10 msec., a mechanical contraction occurs. However, with repetitive stimulation contractions follow with intervals as long as 50 msec. between shocks. The thin (slow contraction) axon requires several stimuli before a muscle action current is set up.

By varying the frequency of stimulation in *Cambarus*, a rate can be found at which stimulation of the fast axon causes maximal muscle action currents

without a mechanical response, while stimulation of the slow axon causes small muscle action currents with a definite contraction. In the first case, the maximal action currents show that all the muscle fibers are being excited, yet no contraction occurs. In the second case, only a few of the fibers are being excited, yet contraction occurs. The action currents in these two cases must arise from different places in the same muscle fiber. The excitation process is therefore a local one.

Van Harreveld (1939a, b) showed that each axon breaks into a "feltwork" of branches which innervate the muscle fiber at a great many points. Slight injury to this network prevents a response, indicating that the stimulus is not conducted over the muscle fiber.

This situation is in sharp contrast to that in the vertebrates, where the action potential, arising at the motor end-plate, spreads over the entire muscle fiber. Local excitation permits a graded response in the crustacean muscle fiber, while the conducted excitation in vertebrates requires an all-or-none response.

Facilitation occurs independently for the muscle action current and for contraction (Wiersma and van Harreveld, 1938b). During stimulation of the slow fiber, the muscle action current at first increases through facilitation, and then declines as fatigue sets in. At the same time the mechanical response is still increasing. Evidently two distinct transmission processes are involved. In one, the nerve impulse sets up a muscle action current, while in the other the muscle action current produces a contraction.

Evidence that the two types of contraction occur in the same muscle fiber comes from several sources. Bergren and Wiersma (1938) showed that the phosphate and lactic acid production in the slow and fast contractions are the same when the mechanical effects are equal. Evidently only one type of contractile substance is present.

Pantin (1934) showed that fatigue of one type of response also fatigues the other, while Wiersma and van Harreveld (1939) found that the mechanical response to stimulation of one motor nerve is facilitated by preceding stimulation of the other nerve. Since this "heterofacilitation" does not occur for the muscle action currents, it is evident that each nerve sets up its own muscle action currents (local response), but that these currents excite the same contractile mechanism.

Finally, van Harreveld (1939a, b) showed that fibers in a triply-innervated muscle receive branches from every axon. Both fast and slow contractions were observed in the same fiber.

The effect of ions on nerve muscle preparations of the crab was observed by Katz (1936a). Excess K causes a contracture, the tension developed in high K solutions equalling that obtained by a maximal stimulation through the nerve. During this contracture the muscle is still excitable to direct stimulation. Excess Mg prevents this K contracture, and also has a curare-like action in blocking neuro-muscular transmission. In the slow fiber of the *Cambarus* claw, Waterman (1941) showed that the amplitude of contraction is inversely proportional to the amount of perfused K. In the fast fiber the opposite re-

lation holds, the amplitude of contraction being directly proportional to the K concentration. In both systems the magnitude of response was inversely proportional to the Mg concentration, while any change in Ca produced irregular results. It is suggested that these ions act on the neuro-muscular junction.

4. *Inhibition.* Two types of inhibition are present in crustacean muscle (Marmont and Wiersma, 1938). If the inhibiting impulse arrives shortly before the exciting one, both the muscle action current and the contraction are reduced. If the inhibiting impulse arrives at any other time, only the mechanical response is reduced. The latter is called "simple", the former "supplemented", inhibition.

Stimulation of the inhibitor nerve alone causes no electrical response in the muscle, but an after-effect is seen by the inhibition of the response to a succeeding excitatory impulse. This persistence of the effect after the nerve impulse has ceased suggests that a neurohumor is involved. Marmont and Wiersma suggest that an "inhibiting substance" may act against a "transmitting substance."

The effectiveness with which excitatory impulses can be inhibited was measured by Wiersma and Ellis (1942). Considerable variation was found between different systems. Thus in the slow bender system of *Pachygrapsus* one inhibitory impulse suppresses three excitatory impulses, while in the slow closer system of *Cambarus* five inhibitory impulses are needed to counteract one excitatory impulse. The fast closer system of *Cambarus* cannot be inhibited; this system also gives a mechanical response to a single stimulus, while the other inhibitable systems do not.

Supplementary inhibition occurs in the stretcher muscle of the *Cambarus* leg but not in the closer, although both muscles are innervated by the same inhibitor. A different situation was found in the crab by Wiersma and Helfer (1941). The opener muscle is supplied by two inhibitor nerves. Stimulation of one inhibits both the electrical and mechanical response, while stimulation of the other inhibits the mechanical response alone. The extent of inhibition is evidently determined by factors in both the nerve and the muscle.

Knowlton and Campbell (1929) found that stimulation of one of two nerves in the lobster claw causes contraction of the closer and inhibition of the opener muscle, while stimulation of the other nerve contracts the opener and inhibits the closer. Van Harreveld and Wiersma (1937) explained this relationship by analyzing the distribution of axons in the nerves of the crayfish (which presumably has the same innervation as the lobster). The thin nerve contains both the axon causing contraction of the opener and that causing inhibition of the closer, while the thick nerve contains axons causing contraction of the closer and inhibition of the opener. This reciprocal innervation of antagonistic muscles differs from that of the vertebrates in that the inhibition is peripheral and not central.

Pantin (1936c) showed that reciprocal responses in the limbs of *Carcinus* are caused by the differences in thresholds between the excitor and inhibitor nerves. A weak stimulus excites the extensor, but has no action on the flexor. A stronger stimulus inhibits the extensor and excites the flexor, while a still stronger stimulus

inhibits both the extensor and the flexor. Such a relation does not occur in mammals.

A more complex situation was found in the crab by Wiersma (1941). All four distal muscles of the leg are innervated by the same inhibitor. Clearly this nerve could not be used in reciprocal reflexes. Wiersma suggests that it may be used in moulting, when relaxation of all muscles is required.

Another explanation is suggested by Pantin (1936c). In the resting limb, normal tone is maintained by low-frequency excitation. A few extra impulses at high frequency will cause a contraction, which is then maintained by the low-frequency discharge. An inhibitory discharge will stop the contraction without interrupting the low-frequency tone. Instead of the continual discharge needed to maintain a vertebrate tetanus, only a few impulses are needed to start this contraction and a few more to stop it. This mechanism enables the crustacean to maintain a sustained contraction at minimum expense.

B. *Arachnida and Insecta.* Little work has been done on arthropod nerve-muscle systems aside from those of the Crustacea. Rijlant (1934) investigated muscle activity in the scorpion. At rest, the flexor muscles show a tonic activity marked by slow action potentials, while there is no activity in the extensors. During contraction, large rapid waves occur in the flexors, while tonic activity appears in the extensors. Flexor tonus is inhibited during contraction of the extensors. In contrast to the mammals, the flexors have a marked tonic activity, while extensor tonus appears only during contraction of the flexors.

Pringle (1939) found that the neuro-muscular system of the extensor tibiae of the cockroach is like that of the crayfish in having "fast" and "slow" contractions produced by stimulation of different axons. Furthermore, the same muscle fiber responds to both types of stimulation. The fast axon has a lower threshold than the slow axon, and produces larger muscle action currents. The chief difference between the two animals is that no evidence for inhibitory fibers was found in the cockroach. The slow axon differs from that of the crayfish in that a single impulse produces a contraction. Further analysis of insect neuro-muscular systems would be of great value.

VI. Pharmacology. The pharmacology of the vertebrate nervous system has taken on a different aspect in the past two decades due largely to the discovery of two physiologically different types of neurons: (1) those whose activity appears to depend on the formation and action of Ach, for which Dale proposed the term "cholinergic"; (2) those characterized by the occurrence of adrenalin for which the term "adrenergic" was proposed. Many drugs used empirically in the past may perhaps act by preventing the rapid destruction of Ach or adrenalin, by substitution, or by blocking the action of Ach or adrenalin at synapses or at neuro-effector junctions. For detailed information one may refer to textbooks on pharmacology (e.g., Gaddum, 1940; Goodman and Gilman, 1941).

In animals other than vertebrates too little is known about the exact distribution of cholinergic and adrenergic neurons, and the mode of action of Ach and adrenalin (as well as the possible occurrence of other "neurohumors"), to enable one to interpret the action of many of the standard drugs which affect

the nervous system. Hence it is quite unsafe to assume that a given drug with a known action in the vertebrates will have a similar action in the invertebrates.

A selected series of drugs, whose action on arthropod nervous systems has been studied, will be considered in this section. Insofar as information allows, their actions on the heart, central nervous system and peripheral nerves will be reviewed. It is difficult in the case of the arthropod heart to determine the exact locus of action of a given drug since the possible loci are several: on cell bodies of pace-maker neurons (when present); on axons of these neurons; at their endings; and directly on the heart muscle. In the intact heart, possessing extrinsic regulatory nerves, the situation is even more complex.

1. *Nicotine.* This active alkaloid of tobacco is well known as an insecticide (Shepard, 1939). In spite of its wide use, however, little is known concerning its exact mode of action in insects. Carlson (1906) applied nicotine separately to the cardiac ganglion and muscle of the *Limulus* heart. When applied to the ganglion nicotine gave initial stimulation followed by irregularities of rhythm, depression, or paralysis, depending on the concentration used. When applied directly to muscle, low concentrations had little effect, high concentrations were depressive. The heart of the crab, *Cancer magister*, (Davenport, 1941) was found to be excited by nicotine in relatively low concentrations and depressed by high concentrations. The action of nicotine on isolated heart preparations of larvae of the southern army worm, *Prodenia eridania*, and of the cockroach, *Periplaneta americana*, was studied by Yeager and Gahan (1937). They observed with both hearts that relatively low concentrations of nicotine caused stimulation, which was not followed by depression, while relatively high concentrations gave initial stimulation followed by complete paralysis. The many differences observed in the behavior of the two hearts to nicotine led these authors to suggest that ". . . the marked differences in response of the roach and larval (*Prodenia*) isolated cardiac mechanisms to perfusing nicotine may be due to differences either in their possession of intrinsic ganglionic cells or in the resistance to nicotine of various of their component or muscular parts". Hamilton (1939) working with the heart of the grasshopper, *Melanoplus differentialis*, found that concentrations of nicotine ranging from 0.0001 to 1.0 per cent by volume caused an increase in amplitude with little change in rate. Repeated applications of 1.0 per cent nicotine caused complete paralysis. Yeager and Munson (1945) have recently compared the actions of nicotine and DDT in *Periplaneta*. Isolated and semi-intact ventral nerve cords of arthropods such as *Cambarus* and *Periplaneta* continue to show electrical activity which is spoken of as "spontaneous". The frequency of discharge is a function of the rate of "firing" of individual neurons, as well as the number of neurons active at a given time. Certain drugs have a marked effect on the level of activity. Roeder and Roeder (1939) found that nicotine 10^{-5} produced an appreciable increase in the spontaneous activity of the isolated ventral nerve cord of *Periplaneta*. Nicotine 10^{-4} produced great activity within fifteen seconds after application, followed in two minutes by complete blocking or paralysis. Prosser (1940) observed similar effects of nicotine on the nerve cord of the crayfish.

In the most complete recent study of the effect of drugs on the peripheral nervous system of arthropods, Ellis, Thienes and Wiersma (1942) observed the action of nicotine on the peripheral nerve-muscle preparation of the cheliped of the crayfish, *Cambarus clarkii*. When nicotine (0.01 mgm./g.) was injected into whole animals there was an initial increase in reflex excitability followed by depression; when nicotine (1 per cent) was applied to peripheral nerve-muscle preparations there was no effect.

The evidence from the several studies suggests that nicotine acts on motor cell bodies and possibly synapses in the arthropod nervous system, causing excitation in low concentrations and depression or paralysis in high concentrations; and it has little or no action on the nerve fiber or at the nerve endings on muscle.

2. *Curare.* In the vertebrates curare has the inhibitory actions of nicotine without preliminary excitation. Its paralytic actions on neuromuscular junctions is much greater than that of nicotine. This action has been shown to be due to a blocking of acetylcholine at motor end-plates. The effects of curare on arthropods are quite different from those on vertebrates but in many respects resemble the effects of nicotine on arthropods.

Carlson (1922) reports that curare, like nicotine, first stimulates and then paralyzes the arthropod heart after cutting extrinsic nerves. In the *Limulus* heart these drugs act primarily on the heart ganglion, not on the heart muscle or the intrinsic motor nerve fibers. No excitatory action of curare on the heart of the crab, *Cancer magister*, was observed by Davenport (1942) but high concentrations brought about periodic stoppage in diastole. Davenport alsofound that previous treatment with curare greatly altered the response of the *Cancer* heart to Ach: either the characteristic excitatory action of Ach was absent or a decrease in rate appeared after curare and Ach.

It has been known for many years that curare acts especially on the central nervous system of crustaceans. Thus Piotrowski (1893) observed that curare injected into a crayfish resulted in complete paralysis but a chela removed showed all of the phenomena of a normal preparation on stimulation of its nerve. Katz (1936a) has made similar observations on the crab, *Carcinus*. Ellis, Thienes and Wiersma (1942) found that the injection of curare (0.3 mgm./g.) into entire crayfish depressed reflex excitability while one per cent curare had no observable action on the peripheral neuro-muscular system of the crayfish. It appears then that in the Crustacea curare has the inhibitory actions of nicotine, usually without preliminary excitation, while both drugs fail to have any demonstrable action on peripheral nerve or myo-neural junctions.

3. *Muscarine.* This drug causes increases in amplitude and frequency of the heart of *Cancer* (Davenport, 1942) but has no peripheral action in the crayfish (Ellis, Thienes and Wiersma, 1942).

4. *Pilocarpine.* Bain (1929) found the isolated heart of *Maia* to be excited by pilocarpine and that this action was blocked by atropine. Roeder and Roeder (1939) likewise observed an excitatory action of pilocarpine on the spontaneous activity of the isolated nerve cord of *Periplaneta;* also that this action could be completely blocked by atropine. Ellis, Thienes and Wiersma (1942) injected

pilocarpine (2 mgm./g.) into entire crayfish and obtained increased reflex activity but failed to obtain an effect on peripheral nerve. With a limited range of concentrations of pilocarpine (5×10^{-4} to 5×10^{-3}) Waterman (unpublished)[3] obtained large amplitude increases in constantly perfused preparations of the flexor dactyl of *Maia*.

5. *Acetylcholine (Ach)*. Much interest has been centered on this highly active substance in the past two decades. It has been shown, with reasonable certainty, to be involved in transmission at ganglionic synapses in the autonomic nervous system; at postganglionic nerve endings in the parasympathetic and at myo-neural junctions of skeletal muscle. It probably plays a rôle in central transmission. Thus far no direct evidence has been presented indicating a rôle of Ach in conduction along the nerve fiber.

The actions of Ach in the vertebrates are commonly classed as "nicotine-like" or "muscarine-like" according to their resemblance to the action of these drugs. For example, the action of Ach on the vertebrate heart resembles that of muscarine; while the action at motor end plates of skeletal muscle resembles that of nicotine.

Arthropod nervous systems contain relatively large quantities of Ach (see section III) but except for its rôle in the control of heart beat the normal functions of Ach in this group are poorly understood. It is important to keep in mind the evanescent nature of Ach in the presence of uninhibited cholinesterase, and the difficulty which must necessarily attend the attainment of appropriate concentrations of this drug at points where it may be expected to act. Dale's group worked with great care and patience to demonstrate that Ach may, when properly administered, produce a normal muscle twitch.

Hearts of vetebrates are inhibited by Ach; the great majority of arthropod hearts are excited. The hearts of all decapod Crustacea that have been studied show an increase in amplitude or frequency, or both, following perfusion with Ach (MacLean and Beznak, 1933; Welsh, 1939a, b, 1942; Davenport, 1941; Davenport, Loomis and Opler, 1940; Wiersma and Novitski, 1942; Prosser, 1942). The heart beat of certain amphipods, isopods and copepods is accelerated by Ach (Prosser, 1942); that of *Daphnia* is slowed (Baylor, 1942), while the beat of the hearts of the primitive crustaceans, *Artemia* and *Eubranchipus* is unaffected (Prosser, 1942). The heart beat of *Limulus* is accelerated by Ach (Garrey 1942). Hamilton (1939) observed an immediate increase in frequency of heart beat in *Melanoplus* following the application of Ach. Prosser suggests that hearts which are accelerated are neurogenic, those which are inhibited are innervated but myogenic, and those which are unaffected are non-innervated. Most observers have noted that atropine abolishes the response of the arthropod heart to Ach. The *Limulus* heart is an exception (Garrey, 1942).

It is difficult to demonstrate an action of Ach on the ventral nerve cord of arthropods at concentrations which are physiologically significant. Bonnet (1938) observed an immediate augmentation in frequency and amplitude of

[3] Waterman, T. H. 1943 Crustacean neuromuscular transmission. (Thesis). Harvard University.

potentials from intact crayfish thoracic ganglia on application of Ach 10^{-9} to 10^{-7}. This effect lasted for one minute and was followed by a depression lasting one to eight minutes, depending on the concentration employed. Concentrations above 10^{-7} were found to have only a depressive action. Prosser (1938) had earlier failed to obtain effects of Ach on spontaneous electrical activity in deafferented abdominal ganglia of the crayfish and following the study by Bonnet carefully repeated his experiments using a wide range of concentrations of Ach and three species of crayfish (Prosser, 1940). He was unable to observe any effect of Ach on spontaneous activity or synaptic transmission in abdominal ganglia at concentrations of 10^{-9} to 10^{-4} inclusive. Higher concentrations did increase the frequency of spontaneous discharge but failed to affect synaptic transmission. Furthermore Prosser (1940) failed to find any potentiation of the Ach action with previous or simultaneous treatment with eserine. Using the isolated nerve cord of *Periplaneta*, Roeder and Roeder (1939) likewise did not obtain an effect on spontaneous activity with either Ach or ethoxycholine until concentrations of 10^{-4} or higher were used. Previous treatment of the cord with eserine 10^{-7}, which is just too dilute to cause an increase in activity, did not increase the response to Ach. Because the cockroach nerve cord responds to low concentrations of such drugs as pilocarpine, nicotine and eserine (Roeder and Roeder, 1939) and contains large quantities of Ach and cholinesterase (Milkalonis and Brown, 1941) it is reasonable to assume that a cholinergic system is playing some part in its normal activity. What this may be, however, remains to be determined.

Several attempts have been made to ascertain whether or not Ach acts as a transmitter substance between motor nerve and skeletal muscle in Crustacea. A variety of techniques has been employed and there has been consistent failure to detect any significant action of Ach on peripheral nerve, or on muscle (Bacq, 1935; Katz, 1936a; Ellis, Thienes and Wiersma, 1942; Waterman, unpublished).

Injection of Ach into intact Crustacea may result in the autotomy of legs or increase the tendency to drop legs which are grasped by forceps (Welsh and Haskin, 1939). The exact mode of action was not determined. It might have been to increase the number of sensory impulses; to facilitate central transmission; to excite motor neurons or to stimulate the autotomizer muscle directly. Ellis, Thienes and Wiersma (1942) observed that injected Ach produced excitation (increase in reflex activity) in the crayfish.

6. *Eserine* (*physostigmine*). This drug acts by blocking the enzyme cholinesterase and allowing applied Ach to act before being destroyed, or allowing an accumulation of Ach at points where it normally acts. The observation that the action of a nerve is increased by eserine may be taken as suggestive, but not conclusive evidence that the nerve is cholinergic.

Restoration of a regular heart beat, and some acceleration with eserine alone, have been observed in decapod Crustacea by Welsh (1939b) and Davenport, Loomis and Opler (1940). Marked potentiation of the action of Ach after eserinization of the decapod heart has been observed (Welsh, 1939b, 1942; Davenport, Loomis and Opler 1940; Davenport, 1941). Comparison of the

acceleration of the crayfish heart produced by maximal stimulation of the accelerator nerve, before and during perfusion with eserine, showed that eserine enhanced the effect of nerve stimulation (Wiersma and Novitski, 1942).

Prosser (1940) found that eserine stimulated cells of ventral ganglia of crayfish to fire repetitively. This action was usually followed by blocking of synaptic transmission. Roeder and Roeder (1939) found that eserine 10^{-4} produced a great increase in spontaneous electrical activity of the isolated nerve cord of *Periplaneta*.

Katz (1936) reported no sign of improved facilitation after eserinization (10^{-6} to 10^{-3}) of crab nerve muscle preparations. Ellis, Thienes and Wiersma (1942), however, observed that eserine increased the tendency of crayfish nerve to fire repetitively. These latter workers observed that perfusion with eserine initiated spontaneous contractions in the isolated chela of the crayfish.

The anomuran crustacean, *Petrolisthes armatus*, was found to be extremely sensitive to injected eserine (Welsh and Haskin, 1939). Injection of 0.3 γ/g. caused increased motor activity followed by recovery; 0.75 γ/g. caused greatly increased motor activity and unco-ordinated movements followed by sluggishness, failure to autotomize and the death of some animals; while 1.5 γ/g. caused extreme activity followed by tetanic convulsions and 100 per cent mortality.

7. *Atropine.* In the vertebrates this drug paralyzes most of the cholinergic nerves with muscarine-like action. It does not prevent the liberation of Ach but apparently blocks "receptor substances" to prevent Ach from acting when liberated. Atropine has, at first, a stimulating action on the central nervous system from the motor area to the medulla, which is followed by depression.

Bain (1929) observed that atropine abolished the excitatory action of pilocarpine on the isolated crustacean heart. That atropine prevents the excitation, produced by perfused Ach of a variety of decapod crustacean hearts has been observed (Welsh, 1939a, b; Davenport, Loomis and Opler, 1940; Davenport, 1941). Davenport (1942) found the action of nicotine on the heart of *Cancer magister* was not in any way modified by atropine; that atropine completely abolished the response to muscarine and acetyl-beta-methylcholine (mecholyl), but that the response to carbamylcholine (doryl) was not abolished by atropine.

Carlson (1906) and Garrey (1942) have observed only an excitatory action of atropine on the cardiac ganglion of *Limulus* and Garrey found no blocking of the excitatory action of Ach after atropinization.

Prosser (1940) found atropine (10^{-4}) had no apparent effect upon the synaptic response in the crayfish nerve cord. Roeder and Roeder (1939) report that the electrical activity of the isolated nerve cord of *Periplaneta* is unaffected by atropine 10^{-3} but that previous atropinization completely prevents the excitatory action of pilocarpine. These latter authors were unable to obtain conclusive evidence that the excitatory action of Ach was blocked by atropine.

Atropine (1 per cent) was found to have an anesthetic action when injected into the isolated chela of the crayfish (Ellis, Thienes and Wiersma, 1942). Its action resembled that obtained with nupercaine, procaine, diothane and amphetamine. Welsh and Haskin (1939) found that atropine injected into *Petro-*

listhes had a depressive action, and, depending on the amount injected, reduced or abolished the autotomy of appendages. Crozier (1922) describes the action of atropine injected into lepidopterous larvae as producing a "reversal of inhibition" since a reversal in the behavior of the prolegs appeared after injection of atropine. Later Crozier and Pilz (1924) obtained general excitation of *Melanoplus* when atropine was injected or applied to thoracic ganglia.

8. *Adrenalin.* This active substance from the suprarenal medulla and adrenergic nerves has many familiar actions in the vertebrates such as augmentation of rate and force of heart beat, inhibition of tone and movement of stomach and intestine, etc. Its actions in arthropods have not been extensively studied. Adrenalin is known to excite the decapod crustacean heart (MacLean and Beznak, 1933; Bain, 1929; Welsh, 1939; Davenport, Loomis and Opler, 1940). Welsh, and Davenport, Loomis and Opler observed that the excitatory action of adrenalin persisted for some time during washing (20 min. or more with isolated heart of *Panulirus*).

Prosser (1940) found adrenalin 10^{-4} to increase somewhat the spontaneous electrical activity of cells in the crayfish ventral nerve cord. Ellis, Thienes and Wiersma (1942) found no effect of adrenalin on the peripheral system in the crayfish but on injection into whole animals there was marked depression of reflex excitability. This agrees with observations made by Welsh and Haskin (1939) on the effect of adrenalin in depressing the autotomy reflex in *Petrolisthes.*

9. *Yohimbine.* This drug in a saturated solution (about 1:20,000) lengthens the refractory period of crustacean nerve (Bayliss, Cowan and Scott, 1935; Marmont, 1941; Ellis, Thienes and Wiersma, 1942).

10. *Veratrine.* This mixture of alkaloids from the underground stems of the white hellebore, *Veratrum*, has a very marked action on peripheral crustacean nerve. Bayliss, Cowan and Scott (1935) found that veratrine 1:25,000,000 when applied to isolated leg nerves of *Maia*, produced a prolonged after-potential which persisted for one hour. Ellis, Thienes and Wiersma (1942) observed that veratrine tended to prolong the refractory period of crayfish nerve but more striking was the initiation of repetitive discharge which produced spontaneous twitching of claw muscle.

So far as is known no study has been made of the action of the veratrine alkaloids on the arthropod central nervous system.

11. *Piperidinomethylbenzedioxane (933F).* This piperidine derivative of dioxane acts in vertebrates to antagonize adrenalin but at the same time stimulates the central nervous system by an adrenalin-like action (Gaddum, 1940). It was found by Waterman (unpublished) to have a powerful excitatory action when perfused through the crustacean appendage. Responses of the perfused flexor dactyl, being stimulated by way of nerve, showed an amplitude increase of as much as 300 per cent. Waterman says, "It was characteristic of the responses to this drug that the contractions became delayed and relaxations slower. This delay was often so great that the tension had only risen to ⅓ of its final value when the burst of stimuli to the nerve was already over. Recovery from these large effects was slow and often incomplete". This action of 933F observed by Water-

man is accounted for by the observation of Ellis, Thienes and Wiersma (1942) that this drug increases the tendency to repeated discharge by crayfish nerve.

12. *Pyrethrum.* Of the insecticides which have not been mentioned in this section there is considerable information on the mode of action of the pyrethrins. The earlier literature is cited by Shepard (1939). Ellis, Thienes and Wiersma (1942) found the pyrethrins, in very low concentrations, when applied to nerve-muscle preparations of the crayfish were among the most effective substances in inducing repeated discharge of the nerve. A single weak shock applied to the nerve after treatment produced a volley of muscle action potentials. Injection of pyrethrins into an isolated claw initiated spontaneous contractions. It is remarked by these authors that spontaneous contractions are especially pronounced with eserine and pyrethrum.

SUMMARY

A selection of a few of the more interesting and characteristic features of the arthropod nervous system follows. The central nervous system consists of a "brain" formed by the fusion of paired ganglia, followed by two ventral ganglionic chains with various degrees of lateral and longitudinal fusion. Sensory neurons have their cell bodies located peripherally. Numerous association neurons are found in the ganglia along with the motor cell bodies which are relatively few in number. Recent studies with the polarizing microscope have shown that arthropod nerve, like that of the vertebrates, is surrounded by a lipoprotein sheath. The carbohydrate reserves of crustacean nerve are much greater than those of vertebrate nerve, as are its oxygen consumption and heat production. It is also more readily fatigued than vertebrate nerve. Evidently the metabolism of crustacean nerve is less efficient than that of the vertebrates. Crustacean nerve usually responds to a single stimulus, unless of very short duration, with a series of impulses. This may be due to its accommodation time being slower than that of vertebrate nerve. Isolated arthropod ventral nerve cords show a persistent rhythmic discharge. Apparently certain cells in the central nervous system discharge without sensory stimulation. Each of the axons innervating a crustacean muscle produces a different type of response. In a triply-innervated muscle, one axon produces a fast contraction, another a slow contraction, while the third inhibits the response to the other two. Since each axon innervates all the fibers of the muscle, the entire muscle acts as a single motor unit. The tension developed is independent of the intensity of the stimulus, and is governed entirely by its frequency and duration. Each muscle fiber receives many branches from each of the axons innervating it. The excitation process is a local one, and is not conducted over the fiber. Hence crustacean muscle shows a graded rather than an all-or-none response. Although arthropod nervous systems contain large quantities of acetylcholine and cholinesterase it has been difficult, by means of pharmacological studies, to demonstrate precise rôles for acetylcholine. Many drugs do not have comparable actions in arthropods and vertebrates. For example, neither nicotine nor curare block transmission from nerve to skeletal muscle in arthropods but both sub-

stances have a central action. A variety of unrelated substances, including veratrine, 933F, eserine and pyrethrum, when applied to peripheral crustacean nerve, produce multiple discharge to single weak stimuli.

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